The Development of Goat Models to Evaluate the Effectiveness of Negative Pressure in Promoting Tissue Ingrowth into Porous Metal Implants

BY

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The Development of Goat Models to Evaluate the Effectiveness of Negative Pressure in Promoting Tissue Ingrowth into Porous Metal Implants

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Abstract

The repair of large segmental bone defects is problematic with a high risk of infection, large amounts of soft tissue damage, and fracture stabilization difficulties. This preliminary study evaluates three large animal caprine models. These models will be used in future studies to examine the effects of negative pressure to induce bone and soft tissue growth into porous metal implants for the repair of large segmental defects.

In Aim #1 bilateral surgery was performed on 6 goats, attaching one large porous titanium implant to the lateral side of each femur. One leg of each animal served as the control while the other side was treated with Negative Pressure Wound Therapy (NPWT) at either -125 mmHg or -200 mmHg. Tissue ingrowth for each negative pressure was examined at 6, 9, and 12 days. After gross examination, 6 of 6 animals showed improved tissue adhesion to the implant treated with NPWT when compared to the implant not treated with NPWT.

Two pilot animals were added to the original protocol in order to explore the iliac crest as a better implant location for soft tissue study. The iliac crest was easier to access, maintain cleanliness of the surgical site, and maintain fixation over the greater trochanter and is recommended as the preferred location for future soft tissue studies.

In Aim #2 unilateral surgeries were performed on 8 goats, replacing a 35 mm diaphyseal segment on the tibia with a porous titanium cylinder (20 mm diameter) and using orthogonal plates to provide rigid fixation. Four goats received no additional therapy and served as the controls while the other 4 goats received NPWT at -125 mmHg directly on the implant for 24-72 hours. Two goats from each group were sacrificed at 6 weeks and 12 weeks and examined qualitatively and biomechanically. Longer durations of therapy need to be examined to determine the effects of negative pressure therapy on bone growth.

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Notations and Conventions (Alphabetical)

12-day animal – length of time between surgery and euthanization for Specific Aim #1 animal

12-week animal – length of time between surgery and euthanization for Specific Aim #2 animal

6-day animal – length of time between surgery and euthanization for Specific Aim #1 animal

6-week animal – length of time between surgery and euthanization for Specific Aim #2 animal

9-day animal – length of time between surgery and euthanization for Specific Aim #1 animal

ACUP – Animal Care and Use Protocol

AFIRM – Armed Forces Institute of Regenerative Medicine

cm – centimeter

EWI – Extremity War Injury

GPa – Gigapascal

H&E – Hematoxylin and Eosin

IACUC – Institutional Animal Care and Use Committee

IED – Improvised Explosive Device

kg – kilogram

mg – milligram

mL – milliliter

mm – millimeter

mmHg – millimeters of mercury

MPa – megapascal

Nm – Newton meters

NPWT – Negative Pressure Wound Therapy

T.R.A.C Pad – suction pad used for negative pressure dressings

V.A.C. – Vacuum Assisted Therapy

Chapter 1 Introduction and Review of Literature

Chapter 1 Introduction and Review of Literature

1.1 Introduction

This pilot study is the initial investigation into the creation of a device that that will apply NPWT across a porous metal implant to enhance the propagation and growth of bone and soft tissue for the treatment of segmental bone defect injuries. This study examines three *in vivo* models to be used in future studies that will evaluate the effectiveness of negative pressure to induce bone and soft tissue growth into porous metal implants. The development of such a device could greatly reduce the reconstruction time of a salvaged limb, improving patient care for those suffering from traumatic extremity injuries. In addition to improving patient care, the outlined research expands the knowledge within the fields of treating large segmental skeletal defects ranging from Extremity War Injuries (EWIs) to osteosarcoma osteotomies, as well as Negative Pressure Wound Therapy (NPWT) and porous metal implants.

The combination of NPWT, with its ability to induce angiogenesis, and newly developed porous metal implants, with similar mechanical properties to bone, may have potential use in the treatment of severe, traumatic segmental defects at an earlier stage in the reconstruction process. The ability of a device to be rigidly implanted at the first definitive debridement and to provide internal fixation in an open and contaminated wound would speed recovery and reduce the number of procedures needed, thereby reducing cost of recovery.

Before the benefits of such a device can be determined, *in vivo* models that can be used to study soft tissue and bone healing in segmental defect injuries need to be established. Until recently, the use of NPWT has been mostly limited to surface applications. This study will also provide valuable information about NPWT and its potential use in closed wounds as well as information about bone and soft tissue growth into porous titanium implants.

The following sections of this chapter provide more in depth, background information about the related topics in order to understand the problem and proposed solution examined in this study.

1.2 Problem: Segmental Trauma Injuries

Peri-articular, open segmental trauma injuries are a prevalent injury and present several challenges for repair. They are often the result of a high impact, traumatic event such as a blast, collision, or fall and are irregular in nature. Infection in such wounds is of major concern due to the open access of dirt and other foreign matter beneath the skin, into the underlying tissues. In addition, because of their location away from the central organs, and priority of healing given to other parts of the body, blood flow is often impaired resulting in decreased healing in extremities leading to nonunion, malunion, and/or compartment syndrome. Tibial shaft fractures are one example of such an injury and alone have an incidence of 17-21 per 100,000 in the population and represent 2% of all fractures in adults [1]. From 1994 to 2003, 10,082 open femoral fractures and 22,479 open tibial or fibular fractures were registered in the American College of Surgeon's National Trauma Data Bank [2].

Figure 1. Extremity war injury with large open periarticular defects

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O'Brien PJ, Cox MW. Stents in tents: endovascular therapy on the battlefields of the global war on terror. J Surg Radiol. 2011 Jan 1;2(1).

Extremity War Injuries (EWI) with large, open segmental defects are another type of open segmental trauma injury, often the result of a munitions round or improvised explosive device (IED) and are a prevalent, high morbidity challenge for the military trauma management

system, presenting all of the challenges previously mentioned. Between October 2001 and January 2005, 1,281 US casualties were reported in operation Iraqi Freedom and Enduring Freedom, of which 75% were caused by explosive munitions [3]. By August 2007, the U.S. had suffered 27,400 U.S. casualties, 24% of which had a fracture and of which 82% were open fractures [4]. In addition to the previously mentioned issues of infection and healing, large amounts of burned or pulverized tissue from a blast makes skin closure and limb repair difficult [4].

Without the same hazard for infection, bone cancers, such as osteosarcoma and Ewing's sarcoma, can be equally traumatic to a patient, leaving a large skeletal and soft tissue defect after surgical removal of the cancer. Although infection due to the presence of foreign matter is not as much of a concern in surgically created segmental defects, repair and healing can be difficult because of a compromised immune system and patient fatigue from additional cancer therapies.

Currently, best practice to reconstruct a limb damaged due to trauma is through multiple procedures that extend over a long period of time. The first step is initial stabilization and debridement of the wound site. Next, external fixation and eradication of infection takes place. Finally, reconstruction and wound coverage takes place often involving multiple procedures. The recovery process for such traumatic extremity injuries can involve months and even years of repeated operations by multiple surgeons at multiple hospitals, often times causing more psychological trauma to the patient than the event that caused the injury [3, 4].

Current practice of immobilizing the fracture by external fixation is not ideal as external fixation is less effective in very proximal appendicular skeletal injuries [4] and can also be cumbersome and uncomfortable for the patient, especially if multiple injuries are sustained, as is often the case [3]. In addition to discomfort, external fixation devices make vascular and nerve repair as well as Vacuum Assisted Closure (V.A.C.) and skin coverage inconvenient.

A better alternative to external fixation is an internal fixation device used in combination with Negative Pressure Wound Therapy (NPWT) that can be used earlier in the reconstructive process. A delay in treatment could be a contributing factor for infection risk. In a retrospective study of 36 open tibia fractures, Cierny *et al.* found a faster rate of healing (4.0 months vs. 6.4 months) and lower frequency of wound healing disturbances (20.8% vs. 83.3%) in tibia fractures that received wound coverage within 7 days of the incident causing the injury [5]. In a similar study examining 38 patients with Gustilo grade IIIB open fractures, patients who underwent definitive coverage with vacuum assisted closure < 7 days post injury were significantly less likely to have infection (12.5%) compared to 57% incidence of infection for patients having coverage > 7 days post injury [6]. Fick *et al.* concluded that conversion to a definitive fixation technique should not be delayed after seeing that infection rates increased with intramedullary nailing proportionally with the time external fixation had been left in place [4]. This evidence supports that earlier definitive fixation and wound coverage is preferred to the current methods of temporary external fixation followed by permanent fixation.

Treating large, open, segmental defects such as EWIs, and other high impact traumatic injuries is a problem for which there are currently no easy solutions to repair large segmental bone defects without multiple surgeries over a long period of time. Advancements in treatment methods and devices to safely, quickly, and effectively treat such injuries are sorely needed.

1.3 Negative Pressure Wound Therapy (NPWT)

Since the early 1990s, it has been known, but not fully understood, that NPWT provides advantages in closing wounds and inducing growth of granulation tissue [4, 6-12]. NPWT is most commonly used in surface applications such as diabetic ulcers or amputations where the patient has negative factors that may compromise normal healing. Factors that increase a patient's risk for poor healing include: advanced age, Diabetes Mellitus, nutritional status, corticosteroid medication, peripheral vascular disease, or tobacco use [8].

NPWT provides several advantages through which it improves healing of health compromised patients. The mechanisms of action that are believed to improve the healing of surface tissue are shown in Figure 2 and include the stabilization of the surrounding tissue, removal of excess fluid, and mechanical stimulation. The removal of excess fluid from the wound is believed to help by removing contamination and waste products from the wound site. NPWT may create and enhance perfusion conditions surrounding the wound. For example, one study showed improved cell growth into porous metals under perfusion conditions [13]. The precise mechanisms of healing caused by mechanical stimulation are unknown; however, mechanical microstimulation and stabilization of the surrounding soft tissue has been shown to be present, as indicated from 3-D computational models [14]. The optimal negative pressure to provide sufficient stabilization of the surrounding tissue, removal of excess fluid, and microstimulation in a surface application has been determined to be -125 mmHg [11]. We chose to take a preliminary look at alternative negative pressures because tissue in a closed wound application may behave differently to negative pressure therapy than the surface wounds it is most commonly used for.

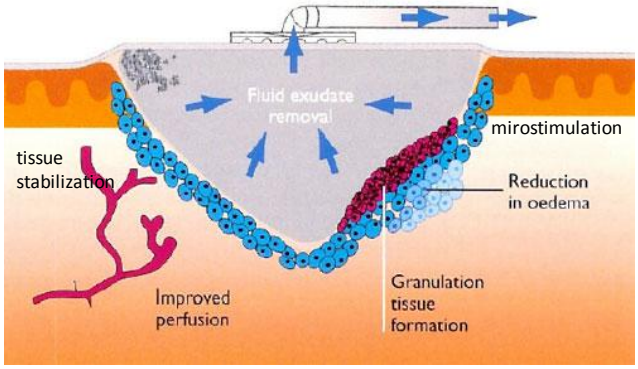

Figure 2. Negative pressure wound therapy diagram of mechanisms of action.

V.A.C.® (Vacuum Assisted Closure) dressings are commonly used in the reconstruction treatment of severe EWIs [3] and facilitate the same advantages when it comes to wound healing as with amputations or diabetic ulcers. The use of NPWT for the treatment of EWIs is mostly limited to injuries requiring skin grafts, and then, only in an open wound, surface application. One reason NPWT has been limited to surface applications has been the question of how deep into tissue the effects (i.e. strain) of negative pressure are effective. One study suggests that

depth of pressure for NPWT is less than 1 mm from the tissue/wound interface [15]. With the depth of therapy relatively shallow, this begs the question of whether there would be enough area affected in a closed wound to make NPWT useful. We will discuss more about the use of a porous metal implant to apply negative pressure in a closed wound in the next section.

While the clinical benefits of NPWT used in surface applications have been well documented, little is known about its effectiveness when used beneath the surface layers of the skin, especially in a closed wound. Recently in 2011, an *in vitro* bench top model along with computer models were used to examine how NPWT relieves stress and strain on the suture line [16]. However, this study did not examine any effects below the layer of the skin. A similar study showed that NPWT improves healing of the dermal layers after 3 days using a porcine model but again this did not show the effects of NPWT on the deep tissue layers of a closed wound [10]. Little has been done to explore the use of negative pressure to treat closed wounds. The lack of knowledge for such an application makes *in vivo* validation of the therapy vital to ensure that the device delivers the expected therapy to the desired location. Even less has been done to examine the effects of NPWT beneath the skin surface making this research vital to our understanding of the effects of NPWT on sub-dermal wound healing.

One possible threat to wound healing caused by NPWT is bacterial bioburden, which can be harbored in a negative pressure dressing. Demaria *et al.* examined the effects of NPWT in the treatment of acute open wounds in 10 dogs. Full-thickness 4 cm by 2 cm wounds were created on the ante-brachium with one side receiving NPWT and the other side receiving standard wound dressings. The wounds were observed and evaluated at 8 time points over a 21 day period. Granulation tissue appeared earlier, was smoother, and was less exuberant in the wounds treated with NPWT. However, prolonged use of NPWT prevented wound contraction and epithelialization giving reason to suggest little benefit in continuing NPWT past 10 days [9]. One

explanation may be that after 10 days, the dressing may have caused an environment antagonistic to healing.

In contrast, a certain increased level of expected bacterial bioburden was shown by Demaria to have non-adverse effects on wound healing. In the previously mentioned study aerobic tissue cultures were taken at 7 and 14 days and, although no animals showed clinical signs of infection over the course of the study, after 7 days bacterial load was higher in wounds treated with NPWT [9]. The normal presence of bacterial bioburden in V.A.C. dressings has also been previously demonstrated in other studies [17].

A similar antagonist to wound healing is the presence of an unregulated air leak in the sealing drape, which resulted in progression of the secondary wound due to dehydration and progressive necrosis [11]. When applied in the wrong manner or for too long, such as in the case with an air leak or without proper perfusion, NPWT can cause an antagonistic environment for tissue growth and wound healing; however, a normal level of bacterial bioburden may be expected in a properly applied NPWT dressing. Overall, when applied appropriately, NPWT has proven to be an effective treatment for healing surface wounds.

1.4 Porous Metals

Relatively new porous metals have demonstrated improved characteristics over solid metal implants, including mechanical strength and biocompatibility, making them well suited for orthopedic applications. A recent study reviewed fabrication methods of porous metals for use in orthopedic applications [18]. Porous metals, such as titanium BIOFOAM™ (Wright Medical Technologies; Arlington, TN), have a decreased rigidity and elastic modulus when compared to the same implant made of solid metal and gives porous metals a more similar rigidity and elastic modulus to that of bone. This similarity in mechanical strength between bone and porous metals could improve fixation issues, such as stress shielding, which is caused by a sudden change in the

rigidity between an implant and the local tissue, making them ideal for providing the structural support required of an internal fixation device used in load bearing applications [19].

However, one concern with using an entirely porous metal implant in a load bearing application is poor fatigue strength as it has been shown that the high cycle fatigue strength of porous coated Ti-6Al-4V alloy is approximately one-third that of the solid alloy of equivalent shape and is probably less than that for entirely porous implants [20]. One way to improve upon the fatigue strength of porous metal might be a post sintering heat treatment which showed a 15% improvement in fatigue properties of porous Ti-6Al-4V coatings [21]. One limitation of this study for the application of an entirely porous metal implant is that it tested a porous Ti coating and not entirely porous implants. If the mode of failure was at the solid/porous interface, an improvement upon this union may not improve the fatigue strength of an entirely porous metal implant without changing its properties.

While the fatigue strength of porous titanium implant itself does not change with bone ingrowth, the fatigue life of the implant and bone construct may be impacted for the better. If bone ingrowth provides union at the bone/metal interface, the bone can share a fraction of the loading, lengthening the fatigue life of the overall construct.

The incorporation of bone and soft tissue into a porous metal implant is ideal both to improve mechanical strength (bone) and to prevent the colonization of biofilms on the surfaces of the implant (soft tissue). The advanced biocompatibility of titanium and other such biocompatible metals allows for proliferation of tissues through the implant. Complete ingrowth of fibrous tissue including vessel formation has been demonstrated in porous tantalum implanted in the backs of dogs [22]. Similarly, 65-70% Ti foam seeded with SAOS (bone tumor cells) and primary osteoblasts demonstrated complete ingrowth of cells through implants in perfusion conditions *in vitro* [13]. If these results can be duplicated *in vivo*, tissue growth throughout the

entire implant is beneficial in order to prevent the invasion of biofilms and/or bacteria in the porous network. This ingrowth also demonstrates potential for bone formation inside of the implant.

A porous metal implant may provide a solution for how to distribute negative pressure therapy to a sub dermal wound. NPWT is commonly used to treat open fractures and encourage soft tissue healing where the loss of skin is prevalent. In a surface wound, a large surface area is available for therapy application whereas the area available in a closed injury compartment is limited. A porous metal implant, such as that made of titanium or tantalum, has a similar geometric structure to that of a traditional sponge. Such a material provides an open porous network with many times the surface area of a solid implant of the same geometry, and allows negative pressure therapy to be distributed through the implant to the surrounding tissue. With this ability to distribute negative pressure while holding a rigid shape, porous metals may provide the solution for how to distribute NPWT in a closed wound to repair segmental defects faster.

As mentioned in section 1.2, there is evidence that earlier coverage of open fractures can decrease risk of infection and speed healing time [4-6]. If an internal fixation device can be combined with NPWT in a closed wound application, such a device could be used earlier in the reconstruction process and improve healing.

In designing a porous metal implant, pore size must be considered so as to provide sufficient mechanical strength and also be optimal for tissue ingrowth. It has been recognized that while pore shape has not been reported to impact biologic response, interconnecting pore size is acknowledged as a critical factor in successful bone ingrowth [18]. An optimum pore size required for implant fixation is currently undefined, but the consensus to optimize mineralized bone ingrowth is that pores between 100 μm and 400 μm are required [23]. However, Bobyn *et al.* demonstrated successful bone ingrowth into porous coatings with pores down to 50 μm and

Itala *et al.* also showed the formation of an osteonal bone structure in pores sizes down to 50 µm [24, 25]. Pore size can directly affect tissue ingrowth and must be considered when designing an internal fixation device made of porous metal.

Several investigators have coined terms to describe the biological events in porous implant healing and stabilization which are useful in the evaluation and understanding of biological responses to porous metal implants [18]. The term *osteoconduction* refers to the situation where bone grows across the surface of an implant [26]. The term *osseointegration* is the rigid fixation of an implant by the formation of bony tissue around the implant without the growth of fibrous tissue at the bone implant interface [26]. Osseointegration is dependent on osteoconduction and maintains fixation of the implant over extended periods while osteoconduction may be short lived. The term *bone ingrowth* refers specifically to bone formation within a porous surface structure [27] and can also be used for growth of bone into an entirely porous implant. Bone ingrowth is dependent on osteoconductive surfaces and will lead to successful osseointegration of the implant.

1.4.1 BIOFOAM™

For this study, Wright Medical Technologies, Inc. (Arlington, TN) manufactured the implants out of their BIOFOAM™ material. BIOFOAM™ is marketed as a cancellous titanium matrix and has similar mechanical properties to that of bone. It was introduced to the market in 2008 as a coating on implants for total knee replacement. Wright's only entirely BIOFOAM™ implant is a wedge system marketed as rigid fixation devices for use in foot and ankle osteotomies.

Wright claims several characteristic material advantages of BIOFOAM™ over other porous metals such as sintered titanium beads and Trabecular Metal™ (Zimmer, Inc.; Warsaw, IN). One such claim is that BIOFOAM™, similar to Trabecular Metal™, achieves 200%-260% greater bone ingrowth than sintered beads at 12 weeks. BIOFOAM™ has a porosity of 60-70%

with a pore diameter of approximately 500 microns [24, 28, 29]. The porosities of BIOFOAM™ and its competitors can be seen in Table 1. The compressive strength of BIOFOAM™ is 86 MPa, which is between cortical bone (140 MPa) and trabecular bone (10-13 MPa) and slightly greater than Trabecular Metal™ (76 MPa) (Table 2). The compressive modulus of BIOFOAM™ is 2.7 GPa, slightly higher than trabecular bone (2 GPa) and much less than cortical bone (15 GPa) (See Table 2). As previously mentioned, having a strength and modulus more similar to that of actual bone is advantageous and prevents stress shielding. BIOFOAM™ also has a coefficient of friction of .55, slightly higher than Trabecular Metal™ (.44), plasma spray (.4), and sintered beads (.32), and allows for more stable fixation than a smooth implant surface (See Table 3). A high coefficient of friction is especially advantageous when trying to achieve natural fixation, without the use of bone cement, where high shear stress can occur, such as the outer surface of an acetabular cup used in a total hip replacement. All material properties for BIOFOAM™ were cited in Wright brochures and the original internal reports could not be obtained from Wright [28, 29].

Table 1. Pore characteristics of porous metals.

	BIOFOAM™ – Wright Medical	**TRABECULAR METAL™ - Zimmer (Implex)**	**REGENEREX™ - BIOMET®**	**GRIPTON - DePuy**	**R2 Acetabular Shells with STIKITTE™ – Smith and Nephew**
Porosity	60-70%	70-80%	67%	63%	60%
Pore Diameter	500 µm	200-600 µm	100-600 µm	300 µm	200 µm
Pore Interconnectivity	interconnected pores	interconnected pores	interconnected pores	Limited Interconnecting Porosity	interconnected pores

Table 2. Compressive strength and compressive modulus of BIOFOAM™, trabecular metal, and bone.

	Cortical bone	**BIOFOAM™ – Wright Medical**	**Trabecular Metal**	**Trabecular bone**
Compressive Strength	140 MPa	86 MPa	76 MPa	10-13 MPa
Compressive Modulus	14 GPa	2.7 GPa	3.0 GPa	2.0 GPa

Table 3. Coefficient of friction or porous metals.

Cofficient of Friction			
BIOFOAM™ – Wright Medical	**Trabecular Metal™ - Zimmer**	**Plasma Spray**	**Sintered Beads**
0.55	0.44	0.40	0.32

1.5 Large Animal Model

A study measuring the peak cutaneous blood flow at varied levels of negative pressure was done on the uninjured arms of volunteer subjects [30]. However, this provided little insight into the actual effects on wound healing as the therapy was applied to a closed skin surface without injury. A live animal model provides the best method of examining the effects of a therapy or method on wound healing. To examine bone and soft tissue ingrowth, an *in vivo* model is required. Birds [31], dogs [9], pigs [11], sheep [32], and goats [33] have all been used *in vivo*.

While pigs are known to have similar vasculature and tissue properties to humans, their limbs are relatively short when compared to human limbs and are not long enough for a segmental defect model. In contrast, birds have dissimilar soft tissue to that of mammals and are not good for soft tissue models of wound healing. Birds also have a small skeletal structure relative to humans.

Goats were chosen for this study because they provide a similar skeletal structure size to that of humans for a large implant and also provide an adequate soft tissue model for examining wound healing. Other groups have successfully used goats to examine the healing of large segmental defects and have established fluorochrome labeling techniques to analyze bone growth [33-35].

1.6 Wound Healing

Soft tissue wound healing has been widely investigated and involves a multistep process to go from wound formation to advancd healing. Initial stages include blood clot formation and inflammation. Inflammatory responses are followed by proliferation and migration of dermal and

epidermal cells along with matrix synthesis to fill the wound gap and reestablish a skin barrier. Lastly, full recovery of skin tissue and restoration of skin aesthetics is achieved through tissue remodelling and differentiation [36].

The growth of granulation tissue reestablishes a skin barrier and involves the proliferation and migration of dermal and epidermal cells. In large segmental defects where deep tissue damage has occurred, a race between granulation tissue formation and the formation of a bacterial biofilm takes place deep inside of the wound. The rapid growth of healthy tissue is extremely important when implants are used to provide fixation, especially porous metal implants, because their surfaces provide an ideal surface for biofilm formation. Once biofilm has colonized and taken over an implant, the only option of treatment is to remove the infected implant. NPWT provides the advantage of sealing out biofilm causing bacteria and could also allow for the infusion of antibiotics to further prevent and fight early stage infection in deep tissue wounds.

When skeletal injuries also occur along with a wound that results in a missing section of bone, this is called a *segmental defect*. A *critical segmental defect* is a defect in the bone that will not heal naturally. The presence or absence of periosteum surrounding the bone may be more important to the determination of a critical size segmental bone defect than the actual size of the defect. In a study attempting to reconstruct large diaphyseal segmental bone defects in goat femurs, it was concluded that the presence of periosteum is essential for the healing of segmental defects [33]. Without the presence of periosteum to allow for revascularization to supply new bone formation, new bone growth cannot take place.

Shallow bone growth into porous titanium implants has been well documented. Porous metals are commonly used as surface coatings on acetabular cups and fermoral implants to achieve biological fixation in hip arthroplasties [18]. However, the maximum ability for bone ingrowth into porous metals remains unknown. While several studies have looked at bone ingrowth, the animal models used have been small and even in the largest animal model, dogs, the implants were relatively small compared to bone size [33]. In that study, limited bone

ingrowth was achieved at 26 weeks if the periosteum was left intact. The maximum depth of bone ingrowth into a porous metal using a large implant begs to be investigated.

Along with missing periosteum, other factors that increase a patient's risk for poor bone healing are the same risk factors that affect soft tissue healing; advanced age, Diabetes Mellitus, nutritional status, corticosteroid medication, peripheral vascular disease, or tobacco use [8].

1.7 Fluorescent Staining of Bone

Fluorochromes are calcium seeking substances that are incorporated into the mineralization front of bone. The florescence of such substances is observed histologically through excitation with ultraviolet (UV) or blue light. There are several groups of fluorochrome labels that include tetracyclines (oxytetracylcine), fluoresceins (calcein), alizarins, and other fluorochromes including xylenol orange. Calcein is generally the preferred fluorochrome label because of its intense visualizations with lower dosing levels. However, multiple labels can be used to observe bone materialization dynamics. The different fluorochromes that are incorporated at the different times of their administration can be used to accurately see the bone growth over time. For instance, Bullens et al. used a calcein green label (20 mg/kg) administered 13 weeks postoperatively and alizarin (30 mg/kg) administered 2 days before euthanization at 26 weeks [33]. Other groups have also shown effective use of multiple label regimens to observe bone growth [33, 37, 38]. Specific Aim #2 utilizes a multiple fluorochrome label regimen to observe bone growth in a segmental tibia defect in goats (Section 3.2.2).

1.8 Histological Processing

Histological processing of porous titanium implants presents several complications over standard histological processing of soft tissues because of their size, porosity and hardness. The implants themselves are larger than small tissue biopsies and because of their porosity, unlike solid implants where the embedding medium need only completely surround the implant, the embedding medium must fully penetrate through an entirely porous implant. If the embedding

medium does not penetrate through the entire specimen, the tissue in the pores will smear during the cutting process. There must not be a sudden transition in structural rigidity between titanium and the embedding medium during the cutting process otherwise the shear forces created by the cutting medium (blade or wire) will cause the different materials to separate. Standard microtome techniques cannot be used to slice through a titanium implant. Instead, a diamond wire saw or Buehler Isomet saw must be used to section a titanium specimen.

To address these complications, histological processes had to be developed in order to analyze the unique implants. To match hardness of the implant, it was suggested that Spurr low viscosity embedding medium be used as the embedding medium [37]. Also recommended was the use of a diamond wire saw that slowly cuts through the specimens, producing little to no heat so as not to damage the specimen.

Several fixatives can be used to preserve the protein structure of tissues for histology including ethanol, formalin, and paraformaldehyde. Ethanol is a common fixative and can also be used for dehydration; however, because it acts through coagulation, it has poor penetration ability and was not a viable option for our larger specimens. Instead, ten percent neutral buffered formalin was recommended as the preferred fixative for this study because of its ability to penetrate into thicker tissue samples [22]. Acetone was also recommended in place of ethanol to dehydrate the specimens because of their large size [37].

Large, porous titanium specimens presented several challenges when it came to histology. Custom protocols had to be developed to examine the bone and soft tissue ingrowth into the large, porous titanium implants.

1.9 Biomechanical Evaluation

Stable fixation is essential to bone healing. A simple gait score has previously been used to evaluate the recovery of animals recovering from a segmental injury and is a good indicator to the stability of the fixation. Unstable implants will not allow the bone to heal and will cause pain

to the animal, not allowing the animal's gait to return to normal. The following gait score system has been used in various studies to evaluate the recovery of animals following surgery [33].

Gait observation scale:

0 = not used at all
1 = supported incidentally
2 = loaded in a standing position and incidentally while walking
3 = loaded in a standing position and while walking but with a limp
4 = normal walking and standing pattern

Natural fixation occurs when bone has grown into the pores of an implant. Biomechanical evaluation is an effective method to evaluate the stability of fixation between both bone and soft tissue to porous metal implants and is somewhat representative of the amount of bone ingrowth. A torsion test has been demonstrated as an effective means of evaluation of bone fixation and a pullout test has been demonstrated as an effective means of evaluation of soft tissue fixation [22, 33].

1.10 Suggested Therapy Solution

At an AAOS Extremity War Injuries III meeting of military injury specialists in 2008, it was agreed that the development of a resorbable bone substitute impregnated with antibiotics and/or growth factors and compatible with internal fixation techniques is desired for the repair of large open segmental defects. Such a device would be withheld until the wound had been eradicated of all infection and it had been determined that the limb would be likely to survive. Used in such a manner, the bone substitute device could reduce the number of procedures needed after its implantation. Current resorbable materials used for an internal fixation device have several disadvantages. Calcium based bone substitutes have the same hazard of fostering osteomyelitis as does bone [34] and non-calcium based scaffolds provide inadequate structural support. Also, depending on if any drugs or growth factors are used, such an implant could have high costs. To overcome these obstacles, this research seeks to combine Negative Pressure Wound Therapy (NPWT) with a porous metal implant, to develop an internal fixation device to

be used in EWI reconstruction. NPWT is known to promote granulation tissue growth and porous meals have similar mechanical properties to bone.

With a porous structure similar to that of a traditional sponge, negative pressure can be distributed throughout the porous network of a porous metal implant using a similar method to traditional NPWT techniques. If negative pressure has a similar effect in a sub-dermal application on bone and soft tissue growth as it does in a traditional surface application, NPWT may be able to be used to repair segmental bone defects more rapidly.

Chapter 2 Specific Aims

Chapter 2 Specific Aims

The objective of this research is to establish *in vivo* models that can be used to examine the effectiveness of negative pressure to induce bone and soft tissue growth into a porous metal implant for the application of segmental bone defect reconstruction. Negative Pressure Wound Therapy (NPWT) is known to promote granulation tissue growth [7, 9, 10, 12, 36] and porous metal implants have similar mechanical properties to that of bone [24, 32, 39]. This preliminary study will move us closer to the development of an internal fixation device and/or treatment to be used in the reconstruction of segmental defect injuries and establish *in vivo* models for the evaluation of such a device.

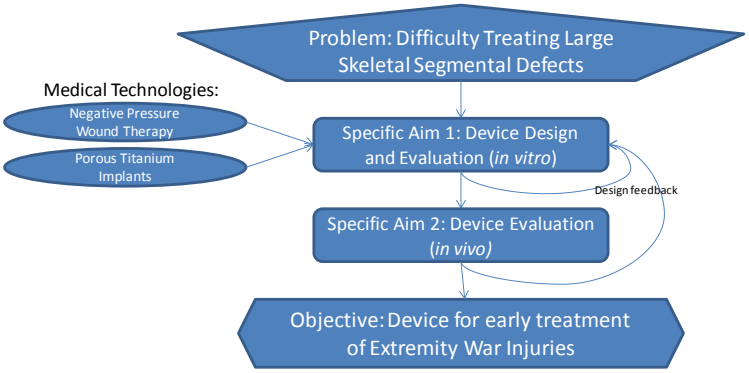

Figure 3. Research flow chart

This research accomplishes this objective through completion of two specific aims. In Specific Aim #1 the granulation tissue block was used to assess effectiveness of NPWT to induce soft tissue growth into the implant at two different implant locations (greater trochanter and iliac crest) using an *in vivo* caprine model. In Specific Aim #2 the tibia segment implant was used to assess effectiveness of NPWT to induce bone growth into the implant using a tibial segmental defect in an *in vivo* caprine model. These *in vivo* models were developed to investigate the hypothesis that NPWT would increase both the amount and speed of bone and soft tissue ingrowth into porous titanium implants.

2.1 Specific Aim #1 – Soft Tissue Growth Models

2.1.1 Specific Aim #1a – Greater Trochanter

Specific Aim #1 evaluated the use of a goat model to determine the effectiveness of negative pressure in promoting soft tissue ingrowth into large porous metal implants. The early

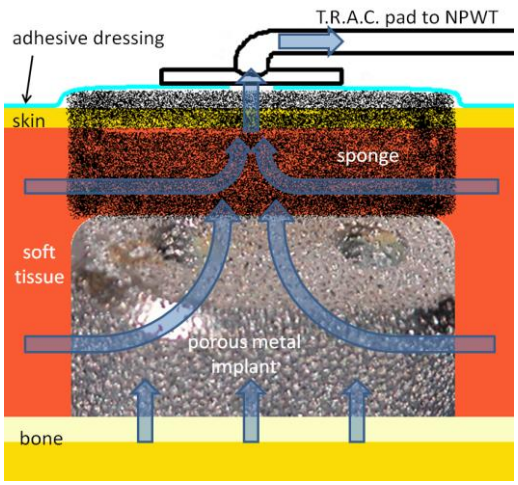

Figure 4. Application of NPWT in Specific Aim #1 with arrows indicating pressure and fluid flow.

formation of soft tissue throughout the implant is an expected precursor to bone formation and beneficial for preventing the colonization of bacteria and the formation of biofilms on the surfaces of the implant. This is advantageous in trying to heal a contaminated wound such as an EWI. To maximize our potential to see a difference in tissue ingrowth, we examined this effect at two different negative pressure levels (-125 mmHg and -200 mmHg) and at three different postoperative time points (6, 9, and 12 days). The implant was attached to the greater trochanter. A diagram of how the therapy was applied in Specific Aim #1 can be seen in Figure 4. Two different levels of negative pressure were examined because of the variance between the sub-dermal application examined in this study and the traditional surface wound application.

2.1.2 Specific Aim #1b – Iliac Crest

Several complications with using the greater trochanter in Specific Aim #1a led to the addition of two pilot animals to test the use of iliac crest as a better site for implantation for examination of soft tissue in a non-load bearing application for future studies. This was not a part of the original study; however, the iliac crest offered potential advantages over the greater trochanter including easier access to the incision site, less threat of loss of implant fixation, and improved cleanliness of the surgical site.

2.2 Specific Aim #2 – Bone Growth Model

Specific Aim #2 evaluated the effect of negative pressure in promoting bone ingrowth into large porous metal implants. Current bone ingrowth is limited to only several mm [23]. Mechanical strength of the construct was examined at two time points (6 and 12 weeks) and bone

ingrowth was assessed histologically and mechanically. It was hypothesized that deeper and faster bone regeneration would occur in implants treated with negative pressure. A diagram of how the therapy was applied in Specific Aim #2 can be seen in Figure 5.

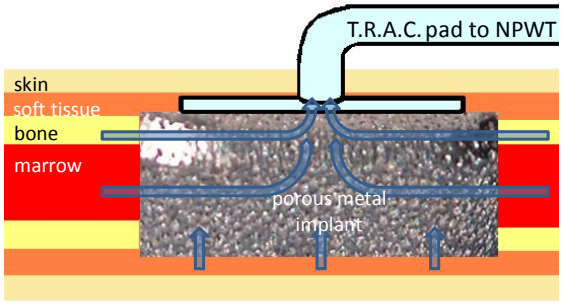

Figure 5. Application of NPWT in Specific Aim #2 with arrows indicating fluid and pressure flow.

Chapter 3 Specific Aim #1a (Soft Tissue Models) – Greater Trochanter

Chapter 3 Specific Aim #1a (Soft Tissue Models) – Greater Trochanter

An in vivo caprine model was used to assess tissue ingrowth. Goats were chosen because of their similar bone size to that of human as well as successful development of the model by other research groups (AFIRM). Six Spanish/Boer cross goats, ages 4-6 and weighing 40-60 kg, were used for Specific Aim #1a. Breakdown of the research groups for each Specific Aim can be seen in Table 4. A soft tissue ingrowth implant to be made of porous titanium implants was designed to deliver NPWT through the implant to the surrounding soft tissue. Design of this implant is described in following section. The Animal Care and Usage Protocol (ACUP), describing the use of animals in this study, was approved by the Institution For Animal Care and Usage Committee (IACUC). The medications used for this study along with their purposes and dosing information can be found in Appendix A.

		Time of Sacrifice		
		6 days	9 days	12 days
Therapy Pressure	-125 mmHg	1	1	1
	-200 mmHg	1	1	1

Table 4. Specific Aim #1 animal groups

3.1 Implant Design (Aim #1) - Tissue Ingrowth Implant

The tissue ingrowth implant was designed to evaluate the effects of NPWT in promoting granulation tissue growth into large porous titanium implants. Wright Medical, Inc. agreed to supply the tissue ingrowth implant, at machining cost, made out of their porous titanium BIOFOAM™ material. The original task described a 35 by 20 by 15 mm implant to be used. A 3-D model of the tissue ingrowth implant can be seen in Figure 6. The ends were rounded and a beveled edge was added to the top of the implant to reduce irritation of the surrounding tissue, which might cause

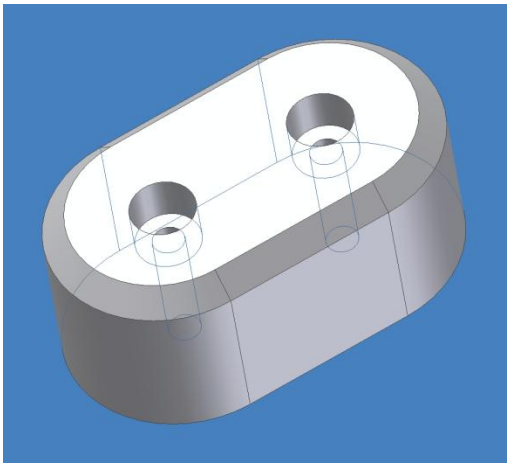

Figure 6. Soft tissue ingrowth implant

an adverse reaction, and affect natural wound healing. Two 2.7 mm diameter counter sunk holes were machined for the two screws used to anchor the implant to the bone. The smallest diameter holes of sufficient length to provide proper fixation to the bone were chosen in order to reduce the amount of interference of the screws with tissue growth inside of the implant.

3.2 Methods (Aim #1a) – Greater Trochanter

3.2.1 Surgical Procedure

Six female Spanish/Boer Cross goats ages 4-6 and weight 30-50 kg were used in Specific Aim #1. Each goat was intubated, anesthetized, and prepared for sterile surgery using aseptic techniques. Site preparation included trimming the hair from the incision area, approximately 15-20 cm circumferentially around the greater trochanter of the femur, and removing any remaining hair from the site using a depilatory (Veet®; Persippany, NJ), applied for approximately 6 minutes. After rinsing away the depilatory and drying, the site was sterilely prepped using an iodine Duraprep sponge stick (Cardinal Health; Dublin, OH) and draped with Ioban (Cardinal Health; Dublin Ohio). Under balanced oral endotracheal anesthesia (isoflurane), each hip was exposed through a 5 cm incision. A thin flake of bone was removed laterally off of the greater trochanter with a power saw to provide a planar surface (See Figure 7). A porous titanium implant was attached to the greater trochanter of each femur of each goat. The porous metal implant, 15 mm by 20 mm by 35 mm (Figure 8) was fixed to the planar surface with two 2.7 mm diameter screws.

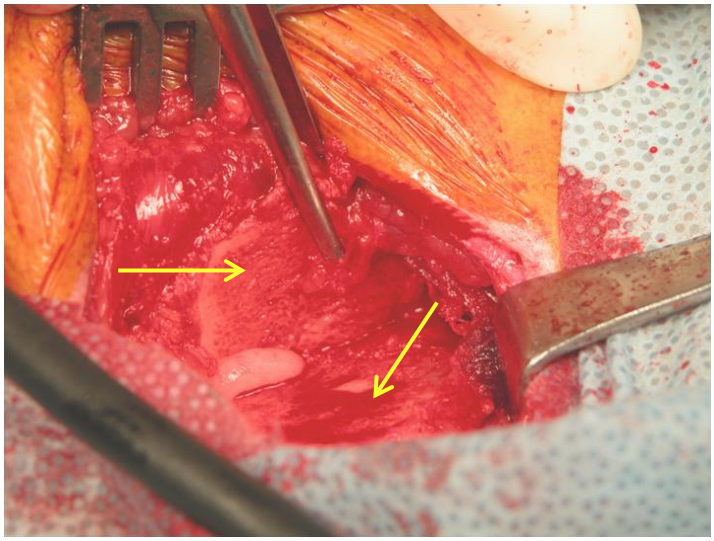

Figure 7. Flake of bone indicated by left arrow. Planar surface for implant indicated by right arrow.

Figure 8. Porous metal soft tissue block used in Specific Aim #1

On one side (control, or no-therapy side) of each animal, the incision was closed over the implant using a 3-0 Vicryl suture. A gauze dressing with a clear adhesive film was applied to dress the incision site.

On the contra lateral side (negative pressure therapy side), a Granufoam® polyurethane sponge (Kinetic Concepts Inc.; San Antonio, TX) approximately 3 cm by 3 cm by 4 cm was sutured to the exposed surface of the implant using 2-0 Vicryl to ensure constant contact between the sponge and implant. The wound was not otherwise closed. A clear plastic adhesive film and suction pad (T.R.A.C. Pad™; Kinetic Concepts Inc.; San Antonio, TX) was applied to cover the sponge using a typical wound V.A.C.® technique as shown in Figure 9. This sterile semiporous adhesive film maintains negative pressure created by the pump. NPWT was applied using a V.A.C. Freedom® system (Kinetic Concepts Inc.; San Antonio, TX) at either -125 mmHg or -200 mmHg. Dressings were changed as needed to maintain an adequate negative pressure seal. One animal of each level of negative pressure treatment was sacrificed at 6, 9, and 12 days postoperatively (see Table 4). Gross observations of tissue adhesion, hematoma, fluid filled bursa, fibrinoid film formation, and fixation to bone were made at necropsy. Specimens were then stored at -80 degrees Celsius to wait histological processing.

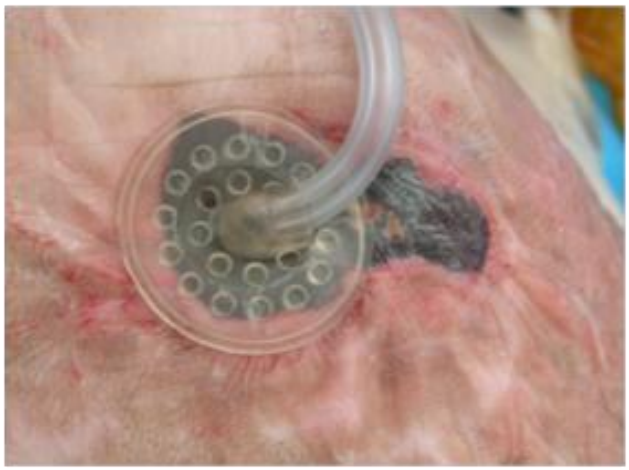

Figure 9. V.A.C.™ Dressing with T.R.A.C. Pad™

3.2.2 Histology

The implants and immediately surrounding tissue were retrieved at the time of animal sacrifice and stored at -80 degrees Celsius until histological processing. Specimens were thawed and immediately fixed in 10% neutral buffered formalin. Following fixation, specimens were dehydrated in 100% acetone and embedded in Spurr's Plastic (Polyscience Inc.; Warrignton, PA) for sectioning. The detailed embedding protocol is shown in Appendix C.

Sections approximately 500 microns thick were taken perpendicular to the length and height of the implant using a Well diamond wire saw (Well Diamond Wire Saws; Norcross, GA). Sections were glued to petrographic glass slides using cyanoacrylate and then ground to ~100 microns thickness using a Buhler grinder with 120, 180, 240, 320, 400 and 600 grit paper. Sectioning and grinding protocol can be seen in Appendix D.

Specimens were embedded and sectioned. The following staining technique has not been completed and will be the focus of further study. Spurr's Plastic will be removed using an Epoxy Resin Removal Kit (Polysciences, Inc.; Warrington, PA) as the initial step in the staining procedure. The slides will be stained using standard Hematoxylin and Eosin (H&E) staining techniques and examined quantitatively to determine the depth of penetration and percent ingrowth of soft tissue for each animal using the non-therapy specimen as the control. The detailed H&E staining protocol can be seen in Appendix D.

3.2.3 Evaluation Methods

As this was a pilot study, the main objective was to establish valid models for the evaluation of the effects of negative pressure on soft tissue growth into large porous titanium implants. The implants for Specific Aim #1 were evaluated qualitatively at the time of implant retrieval for apparent tissue adhesion, film formation, hematoma, infection, fluid filled bursa formation, and final fixation. Qualitative data was evaluated either yes or no for the observed criteria with exception of apartment tissue adhesion. Tissue adhesion was evaluated on a -, ±, +,

++, with (-) being no visible tissue adhesion and (++) being compete tissue adhesion visible on all sides of implant. Other clinical observations were also noted and recorded in the results.

3.3 Results (Aim #1a) – Greater Trochanter

Results were recorded for both Specific Aim #1 and Specific Aim #2. Qualitative results for both aims taken at time of implant retrieval included apparent tissue adhesion, film formation, hematoma, infection, fluid filled bursa formation, and final fixation.

3.3.1 Surgical Procedure and Recovery

Six of six surgeries were executed as planned according to the methods Section 3.1.1. Animal #1 was the first animal to undergo anesthesia and crashed upon intubation. The animal was resuscitated and allowed to recover before attempting surgery a second time. Because of initial complications with the first attempt at anesthesia, the surgery was modified for this animal and the implant was embedded in muscle tissue medial and cranial to the greater trochanter and sutured in place just below the level of the epidermis. A transcutaneous sponge was used to apply NPWT to one of the implants in the same manner as the other groups.

In animal #6, therapy was halted after four days because of inability to reestablish a negative pressure seal after it was initially lost and the open wound was allowed to heal by second intention until sacrifice at 12 days.

Pseudomonas aerugenosa was cultured in goat #3, and gross necropsy findings confirmed infection at 8 days post-surgery. Goats #3 and #4 were sacrificed at 8 and 10 days respectively because of suspected infection.

Fixation was lost in six of twelve implants and was consistent for each animal. For example, both implants maintained fixation or both implants lost fixation for any given animal.

3.3.2 Qualitative Results

Table 5 outlines the qualitative results obtained from the gross observations of the specimens at the time of necropsy for the six animals in the study. Five of six animals

demonstrated improved tissue adhesion to the implant under NPWT compared to the control implant with four of the six therapy implants demonstrating good tissue adhesion. A fibrinoid film formed around three of the control implants while no film formed around any of the therapy implants. Two of six control specimens and one of six therapy specimens had a hematoma in contact with at least part of the implant. A local infection was suspected in two of the therapy implants. It was observed that a fluid filled bursa formed around five of six control implants while no bursa formation was observed around the therapy implants. Fixation was maintained for six of twelve implants and was consistent on both sides for each animal.

Table 5. Specific Aim #1, qualitative results taken at time of implant retrieval.

Animal	Specimen	Actual Time of Sacrifice (days)	Tissue Adhesion	Film Formation	Hematoma	Infection	Fluid Filled Bursa	Maintained Fixation
#1, 6 days at 125 mmHg	No Therapy	6	-	No	No	No	No	No
#2, 6 days at 200 mmHg	No Therapy	6	-	No	No	No	Yes	No
#3, 9 days at 125 mmHg	No Therapy	8	-	Yes	Yes	No	Yes	Yes
#4, 9 days at 200 mmHg	No Therapy	10	-	Yes	Yes	No	Yes	No
#5, 12 days at 125 mmHg	No Therapy	12	±	Yes	No	No	Yes	Yes
#6, 12 days at 200 mmHg	No Therapy	12	-	No	Yes	No	Yes	Yes
#1, 6 days at 125 mmHg	Therapy	6	++	No	No	No	No	No
#2, 6 days at 200 mmHg	Therapy	6	++	No	No	No	No	No
#3, 9 days at 125 mmHg	Therapy	8	++	No	No	Yes	No	Yes
#4, 9 days at 200 mmHg	Therapy	10	++	No	Yes	No	No	No
#5, 12 days at 125 mmHg	Therapy	12	+	No	Yes	Yes	No	Yes
#6, 12 days at 200 mmHg	Therapy	12	±	No	No	No	No	Yes

Supplemental information on each of the six goats in Specific Aim #1.

Goat #1 was a 6-day animal with a negative pressure of -125 mmHg applied to the therapy side. Because of anesthetic complications experienced with this animal during the initial attempt at surgery, the osteotomies of the greater trochanters were not performed and the implants were moved superior and cranial to the femur and embedded in muscle tissue with the implants sutured to soft tissue. Good tissue adhesion was observed on the medial side of the implant subjected to negative pressure therapy. There was no bursa formation on the control side and no apparent tissue adhesion.

Goat #2 was a 6-day animal with the therapy specimen at 200 mmHg. Surgical

procedures were executed according to protocol. It was observed at time of sacrifice that both implants had lost fixation. Optimal tissue adhesion was visible on the therapy side. On the control side, a fluid filled bursa developed around the implant and there was no visible tissue adhesion. Following surgery, the goat experienced decreased rumen mobility from lack of ambulation and a bedsore developed over the control implant.

Goat #3 was a 9-day animal with the therapy specimen at 125 mmHg. The animal was sacrificed at 8 days due to early signs of infection. Fluid cultures taken from the therapy side at time of sacrifice tested positive for *pseudomonas aerugenosa*. The therapy specimen showed visible tissue adhesion while the control side developed a thin membrane and bursa filled with an aromatic fluid consistent of *pseudomonas*. Both implants maintained fixation to bone.

Goat #4 was a 9-day animal with the therapy specimen at 200 mmHg. The animal was sacrificed at 10 days (intended to be a 12-day animal) because of early clinical signs of infection and tested positive for *pseudomonas aerugenosa* although no clinical signs of infection were observed during the dissection. Both implants lost fixation by the time of sacrifice. Optimal tissue adhesion was observed on the therapy specimen. A thin membrane and fluid filled bursa was observed around the control specimen. The observed fluid was not odoriferous.

Goat #5 was a 12-day animal with the therapy specimen at 125 mmHg. Both implants maintained fixation. Some tissue adhesion was visible on therapy specimen and there was no bursa formation or excess fluid. A thin membrane was observed around the control implant, similar to the control specimens on goats #3 and #4, with some tissue adhesion. A fluid filled bursa was observed around the control implant.

Goat #6 was a 12-day animal with the therapy specimen at 200 mmHg. Both implants maintained fixation. Therapy was removed after 4 days of treatment because a proper seal could not be obtained once the original bandage was removed. A fluid filled bursa formed around the

control implant with little to no tissue adhesion. Little tissue adhesion was visible on the therapy specimen and there was no bursa or excess fluid around the implant compartment.

3.4 Discussion (Aim #1a) – Greater Trochanter

Based on the qualitative results from Specific Aim #1a, there does not appear to be a relationship between the use of NPWT and hematoma formation or the maintenance of fixation. Despite small numbers, there may be relationships between the use of NPWT and tissue adhesion, fibrinoid film formation, fluid filled bursa formation, and infection. Loss of fixation in 6 of 12 implants and hematoma formation in 3 implants can most likely be attributed to surgical technique and implant location, which is discussed in the following Chapter 4.

Qualitative results for Specific Aim #1a taken at the time of implant retrieval revealed that 6 of 6 implants showed improved tissue adhesion to the porous titanium implant (See Figure 10 and Figure 11). These results provide preliminary evidence that NPWT may improve tissue adhesion to porous metal implants. NPWT used in combination with an internal fixation device could be promising to speed soft tissue growth to the surfaces of the implant. Rapid soft tissue growth around an implant would be advantageous by preventing the colonization of bacteria on the surfaces of the implant. Of concern however, is the potential for NPWT to create an anaerobic environment in a deep closed wound if excess fluid is not removed. While studies have shown that a level of bacteria can be present inside a negative pressure dressing without causing infection, more evaluation would need to be done to evaluate such use in an infectious model.

One unexpected observation was the formation of a fibrinoid membrane (shown in Figure 10) in three of the control implants. This fibrinoid membrane may also be related to the formation of a fluid filled bursa around 5 of 6 of the control implants in Specific Aim #1 despite different time points or level of negative pressure. This apparent film appeared to seal off the surrounding soft tissue from growing into the porous implant. While the cause is unknown, the fibrinoid film may be attributed to the movement of the surrounding musculature around the implant, further

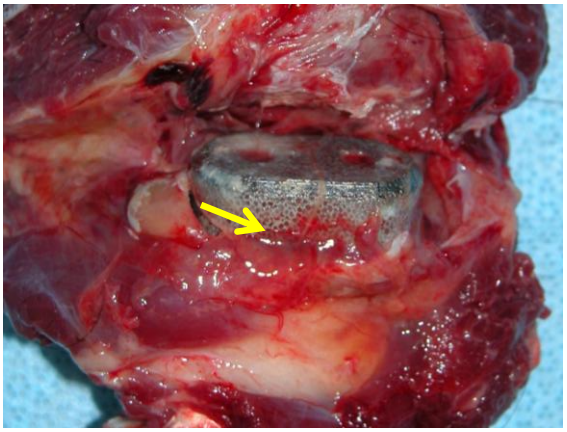

Figure 10. Goat #3, 8 days at 125 mmHg, control specimen. Yellow arrow pointing to apparent fibroid membrane.

Figure 11. Goat #3, 8 days at 125 mmHg, therapy specimen. Yellow arrow pointing to apparent good tissue adhesion.

discussed in Chapter 4. The fibrinoid film can be seen in Figure 10 and good tissue adhesion is shown in Figure 11. Without negative pressure therapy to firmly hold the surrounding soft tissue to the implant, the body may have been protecting itself from the rough surfaces of the implant by covering the implant with this fibrinoid material, so the surrounding soft tissue could freely move around the implant without causing irritation. Three out of five of the implants surrounded by a bursa had a fibrinoid film around the implant as well. Isolation of the implant for more free movement of the surrounding tissue would also explain the bursa formation around five of six of the control implants. We believe that a bursa did not form around the therapy implants because the NPWT initially stabilized the tissue surrounding the implant, allowing tissue attachment and preventing tissue irritation.

Several complications were ascribed to technical problems with negative pressure therapy. The adhesive film did not adhere well to goatskin and hair growth was rapid enough to create air leaks in the dressings after three days. In addition, animal temperament varied making maintenance of negative pressure dressings difficult with some animals, as was the case in goat #6. Finally, maintaining cleanliness of the dressings and implant fixation was difficult due to implant location.

To obtain initial sealing of the adhesive dressing, the surgical site surrounding the implant had to be shaved and a depilatory used to remove excess hair. In addition, Adapt® stoma paste was used to seal any creases that may have been in the dressing. After seven days, sufficient hair growth had taken place to interfere with adherence of the adhesive drape making continued application of NPWT difficult without redepilatating the skin around the incision. For this reason, a less hairy animal, possibly a pig, would be preferred to examine NPWT in the future.

In addition, animal temperament in reaction to the NPWT made initial recovery difficult. Some animals were up and walking normally within hours of surgery while others were not so quick to recover. Whether from the surgery itself, the harness that was used to carry the pump, or stress, some animals were not as eager as others to walk following surgery and this resulted in poor rumen and digestive function. To combat the decreased digestive function that was seen in Specific Aim #1, a probiotic was used in Specific Aim #2 and is recommended following any surgical procedure or other stress related activity such as transport of ruminants.

The use of NPWT also appeared to have an increased incidence of infection. The two infections were both suspected in the therapy implant. Infection however, may have little to do with the therapy itself and more to do with maintaining cleanliness of the open wound in an animal model. Local infection was suspected in two of the therapy specimens in Specific Aim #1 with one confirmed by clinical observation and culture. With the implants placed on the femur, the animal was able to lay in its own feces and urine, soiling the bandages and dressings. Maintaining hygiene around the surgical site is one difficulty involved with using an animal model. One would not expect simple care and cleaning of a surgical site to be such a hazard if a similar therapy were used in a human application. For hygienic reasons and maintaining a clean bandage, it is recommended that the location of future soft tissue studies be moved from the femur to the iliac crest. Advantages include easier access for the changing of bandages as well as

ease of maintaining cleanliness of the bandages and dressings. This alternative location is discussed more in Chapter 4.

Poor cleanliness of the surgical site may have led to infection; however, accurate detection of early infection was difficult in these animals for both Specific Aim #1 and Specific Aim #2. With different temperament between animals, it was difficult to tell if an animal was showing signs of distress as a result of the surgical procedure or from an early infection. Swab cultures proved ineffective as there was a normal environmental presence of *pseudomonas aerugenosa* that would result in a positive culture whether or not an animal showed any clinical signs of infection. This leaves one infection in Specific Aim #1 and one infection in Specific Aim #2 in question, both of which showed no clinical signs of infection. The one animal in the therapy group of Specific Aim #2 that was euthanized at 3 weeks due to infection developed an open lesion that produced pus, an obvious clinical sign of infection. Upon opening the incision along the plate, the infection ran the length of the plate with pus. Despite the infection, after an initial spike in temperature of 103.5 $^{\circ}$F the day the lesion was first noticed, the temperature dropped back to within normal the following day. The goat was able to compartmentalize the infection and probably could have survived much longer; however, the implant had been compromised at that point and the animal was euthanized according to protocol. Better methods to monitor for infection would be of benefit in future studies.

Fixation failure in half of the implants (6 of 12 implants) in Specific Aim #1 was ascribed to small screw diameter, constraint of screw placement, and implant location. Bone quality at the chosen site was less than expected when the implant was designed. This made small diameter cortical screws somewhat ineffective for the location that was used. In addition, location of the femur on that lateral side of the animal may have attributed to loss of fixation in some cases. The implants protruded from the lateral surface of the femur making it easy for the goats to fall or lay on the implants, which in combination with the small screw diameter may have caused loss of

fixation. In the future, a larger diameter screw should be used to improve the overall strength of fixation.

While multiple complications occurred including maintaining fixation, maintaining cleanliness (related to infection), and movement of the surrounding soft tissue, these are all implant site related. To resolve these issues, we proposed changing the implant location for the study of soft tissue from the femur to the iliac crest. Two animals were piloted using the iliac crest and produced better results with fewer complications. These two pilot animals and how the iliac crest provides a better location for a soft tissue, non-load bearing study are discussed in detail in Chapter 4.

Chapter 4 Specific Aim #1b (Soft Tissue Model) – Iliac Crest

Chapter 4 Specific Aim #1b (Soft Tissue Model) – Iliac Crest

Multiple concerns arose during the study for Specific Aim 1 created by the location of the implant as well as the unpredictability and variability of the temperament of each animal. Three animals lost fixation of both implants which could have been created by a high impact force, such as falling on their hip/implant following surgery. Loss of fixation also could have occurred from lower impact forces, such as repeatedly laying on their hip and putting pressure on the implant. In addition to implants losing fixation, the proximity of the incisions to the perineum of the animal and the ground when lying down increased the risk for infection of the incision site. Lastly, the muscle surrounding the femur is very mobile during ambulation causing movement of the tissue across the protruding implant. The movement of the muscle tissue across the implant surface is not ideal and could have been a contributing factor to observed bursa formation.

In response to these concerns that arose from Specific Aim #1, two pilot animals were completed in parallel with Specific Aim #2. The purpose of these 2 pilot animals was to test the feasibility of using an alternative location of the iliac crest for placement of the granulation tissue block implant.

4.1 Methods (Aim#1b) – Iliac Crest

For these 2 goats, the IACUC approved an addendum to the original protocol. All presurgical, surgical, and postsurgical procedures remained the same as those of Specific Aim #1 with exception of the implant location. The implant location was moved from the greater trochanter of the femur to the iliac crest (See Figure 12). NPWT was applied in the same manner as specific Aim

Figure 12. Bilateral iliac crest before closure on the control side and application of negative pressure on the treatment side.

#1a. Negative pressure dressings were changed every three days. One animal was sacrificed 2 weeks after surgery and the other animal was sacrificed 4 weeks after surgery.

4.2 Results (Aim #1b) – Iliac Crest

Gross observation results of the iliac crest were favorable and presented multiple advantages over using the femur. Because the iliac crest is where many muscles are anchored, there is less mobility of the surrounding tissue. With stationary anchor points surrounding the implant, the iliac crest provides a more favorable environment for soft tissue growth into the implant. The lack of mobility of the surrounding tissue as well as not having to lie on one of the implants also appeared to cause less discomfort to the animal. The location is higher off of the ground and away from the perineum of the animal improving cleanliness of the surgical sites and also making dressing changes easier. The animals exhibited no signs of infection. Lastly, the dorsal location of the implants means that the animals cannot fall or lay on the implants. Both implants maintained fixation for the entire 2 or 4-week duration of the study.

In the iliac subjects and the gluteal subject without screws, better responses to therapy were noted than in the trochanteric subjects. The 6-day gluteal subject showed better soft tissue attachment than did the trochanteric ones at longer intervals. This was ascribed to less gliding of the skin and soft tissue over the more prominent trochanteric implant.

The 2-week iliac subject showed the most clearly defined contrast between the open wound with therapy, and the uncomplicated closed wound with no therapy. The no therapy side showed a dark red-brown triangular section mass just lateral to the periosteum, about 1.5cm on each side. This was grossly consistent with edematous muscle and/or organizing hematoma. The same area on the open wound/therapy side showed dense, dry, white early scar without inflammation.

The 4-week iliac goat showed a similar degree of maturation of the soft tissue attachment between the therapy and no-therapy sides. There may or may not have been some skin ingrowth

on the open, therapy side (See Figure 13). There was no bursa formation on the no-therapy side. The gross appearance suggested that the no therapy side had "caught up" with the therapy side at the 4 week interval.

4.3 Discussion (Aim #1b) – Iliac Crest

One limitation of the two animals that were piloted using the iliac crest as the implant location was how the implants were closed. The implant on the control side was completely closed over while the therapy implant remained transcutaneous in order to apply negative pressure therapy. To overcome this for future studies, an alternative method of negative pressure therapy application would need to be developed in order to maintain consistency of closure over the implant.

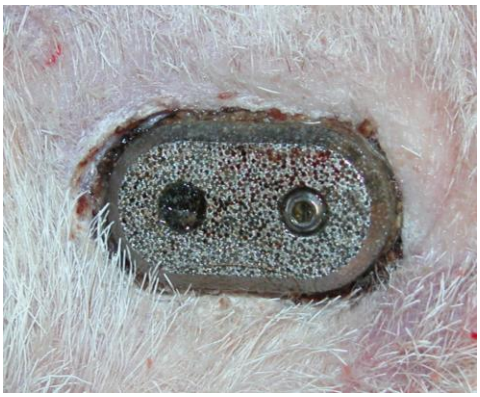

Figure 13. Ingrowth implant applied to iliac crest and transcutaneous with negative pressure therapy for 4 weeks. Note open pores and skin attachment.

Based on the observations of these 2 animals, the iliac crest is a superior location for this type of study and is recommended for future studies of soft tissue growth into large porous metal implants.

Chapter 5 Specific Aim #2 (Bone Growth Model) – Tibia

Chapter 5 Specific Aim #2 (Bone Growth Model) – Tibia

An in vivo caprine model was used to assess bone ingrowth. Goats were chosen because of their similar bone size to that of human as well as successful development of the model by other research groups (AFIRM). Ten Spanish/Boer cross goats, ages 4-6 and weighing 40-60 kg, were used for Specific Aim #2. Two goats served as pilot animal to evaluate the surgical procedure and husbandry needs and the remaining 8 goats were divided evenly between the therapy and control groups. Breakdown of the research groups for Specific Aim #2 can be seen in Table 6. A tibia segment implant to be made of porous titanium implants was designed to deliver NPWT through the implant to the surrounding soft tissue. Design of this implant is described in following section. The Animal Care and Usage Protocol (ACUP), describing the use of animals in this study, was approved by the Institution For Animal Care and Usage Committee (IACUC). The medications used for this study along with their purposes and dosing information can be found in Appendix A.

	Time of Sacrfice	
	6 weeks	12 weeks
Control	2	2
Therapy	2	2

Table 6. Specific Aim #2 animal groups

5.1 Implant Design (Aim #2) – Tibia

This section describes the design process for the first two implant designs that were piloted and how we decided on the implant that was used for the study.

The tibia segment implant was designed to evaluate the effects of NPWT in promoting bone and soft tissue growth into porous titanium implants. Wright Medical, Inc. agreed to supply the tibia segment implants, at machining cost, made out of their porous titanium, BIOFOAM™ material. The original design specified a 25 mm diameter by 35 mm tall cylinder.

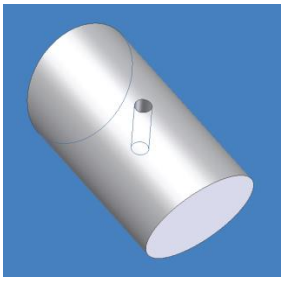

Figure 14. Tibia segment implant.

A 3-D model of the first tibia segment implant can be seen in Figure 14. The planes of

both ends were inclined 15° in order to increase compression between the bone/implant interfaces as the screws of the construct are tightened. In addition, the inclined planes prevent the implant from sliding out from between the two bones on the opposite side of the plate. A screw hole was also added to the middle of the implant for a screw to hold the implant against the plate and align the implant rotationally. For the second pilot animal, a 4 mm diameter through hole was added the length of the implant (Figure 15) for a channel drain. This change would more directly apply suction through the porous metal implant.

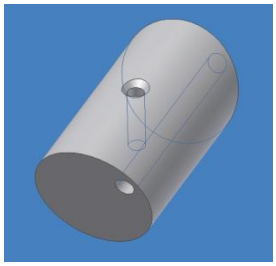

Figure 15. Tibia segment implant with through hole.

Hardware for the tibia segment procedure was purchased from Veterinary Orthopedic Implants (VOI). For the two pilot procedures, a 6 hole, 14 mm width, compression plate was chosen. This width of plate was recommended by VOI for the size of goats chosen for this study. This fixation method, using only 4 screws, was deemed insufficient after two unsuccessful bilateral pilot animals. The middle two holes were covered by the implant and therefore not used. A hole was added in the center of the plate for a screw to hold the cutting guide in place and, once the implant was in place, for a 16 mm long, 4.5 diameter screw to screw the implant to the plate.

For the surgery, 4.5 mm diameter screws ranging from 22 mm to 36 mm were made available, 4 of which were used for each tibia based on measurement with a depth gage. For the first pilot animal, self-tapping screws were used which added time to the surgery and were deemed inadequate. A tap, purchased from VOI, was used in the second pilot improving ease of surgery and decreasing operation time.

Also needed for the tibia procedure was a cutting guide to assist in making accurate, reproducible angled cuts to properly fit the implant. A prototype was machined in the lab out of aluminum and bench tested before was a final guide was machined out of stainless steel by an

outside machine shop. The cutting guide was designed to be screwed into the bone over the plate while the cuts are being made. A 3-D image of the cutting guide can be seen in Figure 16. Figure 17 shows the cutting guide in use on a goat tibia.

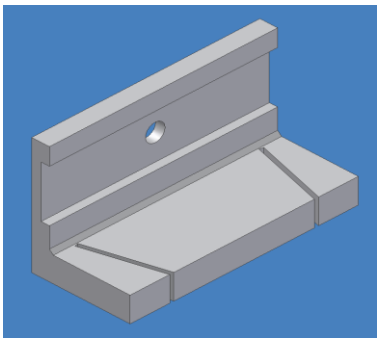

Figure 16. Tibia segment cutting guide.

Following two unsuccessful pilot surgeries, one from anesthesia complication and the other due to the animal's inability to walk following surgery from the bilateral procedure, the one plate method using fours screws was determined to provide inadequate fixation. The implant was redesigned to be a traditional cylinder, 20 mm diameter by 35 mm long (Figure 18) in order to reduce the complexity of the surgery and a new fixation technique using two plates in an orthogonal arrangement (Figure 19) was used to improve stability. A machine screw (8-32) instead of a bone screw was also used to hold the implant to the long plate. This method is described in detail in the surgical methods Section 5.2.1.

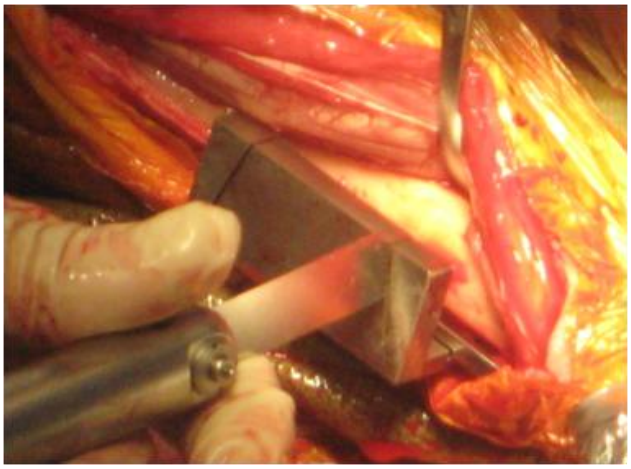

Figure 17. Cutting guide in use.

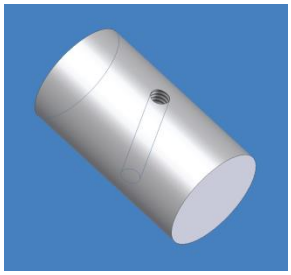

Figure 18. Final tibia segment implant design, straight cylinder.

5.2 Methods (Aim #2) – Tibia

5.2.1 Surgical Procedure

Eight female Spanish/Boer Cross goats ages 4-6 and weight 30-50 kg were used in Specific Aim #2. All orthopedic screws and plates were purchased from Veterinary Orthopedic Implants (V.O.I.; St. Augustine, FL). A list of surgical instruments, hardware, and supplies can

be found in Appendix E. A center hole was added to the 12-hole plate to attach the implant to the plate using a stainless steel machine screw (8-32). This hole with machine screw can be viewed in Figure 19. All screw lengths were measured with a depth gage and rounded up to the nearest 2 mm.

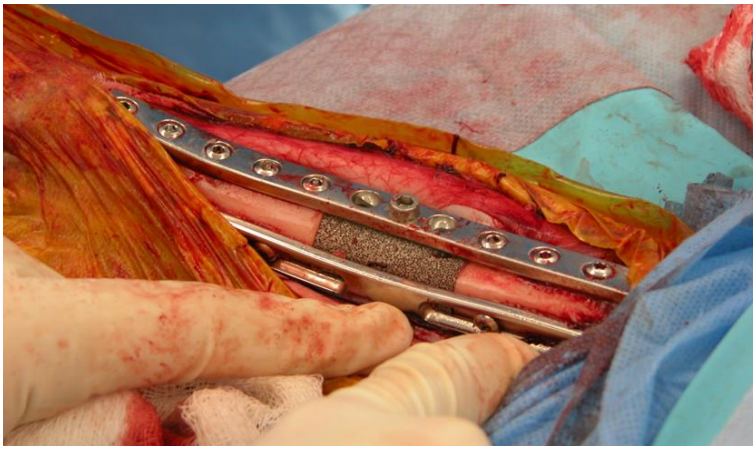

Figure 19. Completed tibial osteotomy construct

Each goat was intubated, anesthetized, and prepared for sterile surgery using aseptic techniques. Site preparation included trimming the hair from the incision area and removing any remaining hair from the site using a depilatory (Veet® Parsippany, NJ), applied for approximately 6 minutes. After rinsing and allowing to dry, the site was sterilely prepped with an iodine Duraprep sponge stick (Cardinal Health; Dublin, OH) and the leg sterilely draped with Ioban (Cardinal Health; Dublin Ohio). The side that was chosen for the procedure was alternated with each surgery.

Under balanced oral endotracheal anesthesia, one tibia was exposed through a medial incision approximately 10 cm in length beginning just proximal of the distal joint of the tibia. The periosteum was circumferentially separated from the tibia along the tibia shaft with a Cobb Elevator. The porous metal implant, 20 mm diameter by 35 mm length (Figure 20) was soaked in a heparin solution of 10 mL of saline mixed with 1 mL Heparin (10,000 units/mL) to keep the implant patent throughout the duration of the surgery.

The 12-hole plate was fitted to the medial surface of the tibia using plate benders when necessary. A bicortical screw was drilled, tapped, and tightened in the center hole of the 12-hole plate (a countersunk center hole was added to the center of the plate to fit an 8-32 machine screw used to secure the implant to the plate). Pockmarks were made in the 4th hole from center on both sides of the plate to be used for alignment following the osteotomy. The slotted plate was aligned on the posterior surface of the tibia and two screws in the proximal hole and two screws in the distal hole were drilled, tapped, and loosely tightened being careful to avoid the screw holes in the 12-hole plate and where the implant would go.

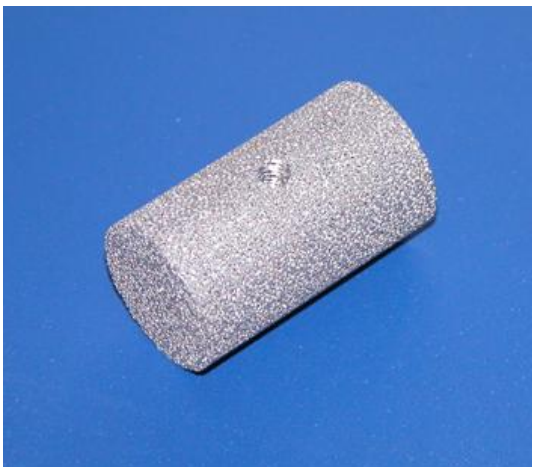

Figure 20. Porous metal tibial implant used in Specific Aim #2

All but the most proximal screw in the slotted plate was removed. The slotted plate was rotated out of the osteotomy site and the cutting guide was screwed into place with a bicortical screw. After aligning the cutting guide with the 12-hole plate, a second screw was put into the guide to prevent rotation during sawing. The osteotomy was performed using a power saw; the distal cut was followed by the proximal cut, being careful to stay alligned with the cutting guide.

The slotted plate was then realigned and the four screws were loosely screwed into the previously drilled holes. The implant was loosely screwed to the 12-hole plate using a ¾", 8-32 stainless steel machine screw and aligned using the previously drilled pockmarks. A screw was eccentrically drilled, tapped, and tightened in the third hole proximal from center to apply compression between the proximal segment of bone and implant interface. Screws were centrically drilled, tapped, and tightened in the 4 remaining unused proximal holes.

The distal end of the tibia was aligned with the 12-hole plate and a Verbrugge clamp was used on the screws in the slotted plate to compress the interface between the distal bone and the implant. If necessary, a second Verbrugge clamp was used to compress the distal end of the plate to the tibia. With compression applied, a screw was eccentrically drilled, tapped, and tightened in the third hole distal from center to apply compression between the distal segment of bone and the implant interface. With compression still applied, the most proximal and distal screws in the slotted plate were fully tightened. After removing the Verbrugge clamps, the two remaining screws in the slotted plate were fully tightened. Screws were centrically drilled, tapped, and tightened in the 4 remaining unused distal holes.

All 8 tibiae were prepared in the previously described manner. The animal groups for Specific Aim #2 can be seen in Table 5. For the four control animals, a channel drain was placed next to the implant and the incision was sutured closed with 3-0 Vicryl in a running suture pattern. The drain exited the body through a poke hole approximately 3 cm posterior of the incision and the drain tube attached to an external bulb to remove excess fluid for 48 hours or until the wound stopped producing fluid.

For the 4 therapy animals, a T.R.A.C. Pad™ was placed under the periosteum on the lateral surface of the implant. The T.R.A.C. Pad™ was secured on the posterior-lateral side of the implant with two 2-0 Vicryl sutures that went around the implant connecting both sides of the T.R.A.C. Pad™. The tube was brought through the skin through a poke hole approximately 3 cm posterior of the incision. Two interrupted sutures were used to close the poke hole around the tubing. The incision was then closed using a 3-0 Vicryl suture in a running suture pattern, providing an airtight seal. Negative pressure therapy was then applied to the implant using a Freedom V.A.C. Freedom® (Kinetic Concepts Inc.; San Antonio, TX) system at -125 mmHg for 3 days or until therapy was no longer feasible. Therapy times varied within this time period (24-72 hours) in order to determine how long negative pressure therapy could be maintained when

applied in this manner. Coagulation of blood in the tubing would cause the pump to shut down when negative pressure could no longer be maintained. Pump error messages along with troubleshooting methods can be seen in the V.A.C. Freedom® Alarm Troubleshooting Quick Reference guide in Appendix F. The goats recovered from anesthesia in a partial weight bearing sling (Figure 21) until the animals regained leg control. The pump was carried by the animal in a custom harness that can be seen in Figure 22. Two animals for each group, therapy and control, were sacrificed at each time point, 6 and 12 weeks (see Table 6).

Figure 21. Partial weight bearing sling used during animal's initial recovery from surgery.

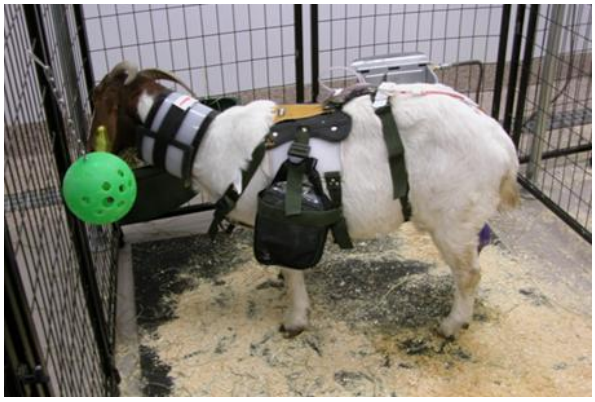

Figure 22. Goat harness used to carry pump.

Each animal underwent the fluorescent staining regimen described in Section 5.2.2. Throughout the time period of the study, each animal's gait was graded with the following gait score [33]; 0 = not used at all, 1 = supported incidentally, 2 = loaded in a standing position and incidental while walking, 3 = loaded in a standing position and while walking but with a limp, and 4 = normal while walking and standing. Animal records that were kept following surgery include gait, medications, weight, therapy duration, temperature, and pre-surgery physical vet approval. Observation sheets can be seen in Appendix G. At the time of necropsy, tibia constructs were evaluated grossly for bone growth, tissue adhesion, hematoma, fluid filled bursa, fibrinoid film formation, and fixation to

bone. Tibia constructs were then mechanically tested according to the procedure described in Section 5.2.3 and will undergo histological analysis as a part of further study.

5.2.2 Fluorescent Staining Regimen

Every animal in Specific Aim #2 underwent a fluorescent staining regimen for the duration of the study. Three fluorescent stains, calcein, oxytetracycline, and xylenol orange were chosen for this study based on staining characteristics. Stains were administered every 2 weeks via injection according to the protocol. Animals sacrificed at 6 weeks received an injection at 2 and 4 weeks while animals sacrificed at 12 weeks received an injection at 2, 4, 6, 8, and 10 weeks. Mixing directions for the calcein and xylenol orange injection are given in Table 7. Oxytetracycline is a common antibiotic and was purchased in injectable form. Dosing instructions for each stain are given in Table 8.

Table 7. Fluorescent stain injection preparation mixing directions

Calcein Green (Sigma-Aldrich, Product #C0875)	
To make 50 mg/mL solution:	
Total Amount:	10 mL
Calcein (Sigma # C0875)	500 mg
NaHCO3 (Fisher #233-500)	500 mg
Sterile Saline	10 mL
Directions: Stir covered until dissolved. Store at 4° C in the dark for no more than 1 week. Remove from refrigeration ½ hour before administration.	
Xylenol Orange ACS reagent (Sigma Aldrich, Product #398187)	
To make 90 mg/mL solution:	
Total Amount:	50 mL
Xylenol (Sigma # C0875)	4500 mg
Sterile Saline	50 mL
or 1.4% isotonic NaHCO3	50 mL
Directions: Dissolve in physiological saline or in 1.4% isotonic NaHCO3 at a concentration up to 90 mg/mL. Can be stored at 4° C for several weeks.	

Table 8. Fluorochrome stain recommended dosing

Stain	Solution Concentration	Dosing	Volume (50 kg animal)	Method of Administration	Administration Time
Calcein Green	50 mg/mL	10-30 mg/kg	10-30 mL	SQ	2 weeks and 8 weeks
Oxytetractcline (yellow)	200 mg/mL	20-25 mg/kg	6.25 mL	IM	4 weeks and 10 weeks
Xylenol Orange	90 mg/mL	90 mg/kg	50 mL	SQ	6 weeks

5.2.3 Mechanical Testing

All specimens in Specific Aim #2 were tested for biomechanical strength using a similar method to the one found in the study performed by Bullens *et al.* [33]. After the removal of all excess tissue from the tibia, the ends of both the operated and contra-lateral tibiae were embedded in polymethyl methacrylate (Patterson Dental, St. Paul, MN) so that the former defect located in the center was free with a margin of 3-cm on the proximal and distal sides of the osteotomy site. The 12-hole plate was removed before potting. The slotted plate was left in place, and its screws were removed just before testing to prevent damage to the construct during the potting process. The specimens were mounted on a Materials Testing System machine (MTS System Corporation; Eden Prairie, MN) so that external torsion was applied to the distal end while the proximal end was free to translate on an x-y table. The x-y table was used to compensate for any off axis alignment. A "zero" load control was used to maintain an axial force of 0 N during the duration of the test. All tibiae were tested for load to failure at a rate of 2 degrees per second to simulate a dynamic loading condition [40]. Torque at failure (maximum torque) was selected to reflect torsional strength and was expressed as a percentage of torque at failure (maximum torque) relative to the contra-lateral (non-operative) leg of the same animal. Therefore, a percent torsional strength of 100% is the same torsional strength as the non-operative leg. In order to preserve the specimens for histological testing, mechanical testing was automatically stopped immediately after fracture or failure indicated by a clear audible sound and drop in mechanical strength to 40% or less. A potted and mounted tibia is shown in Figure 23.

Figure 23. Biomechanical testing setup.

All mechanical testing was performed within 36 hours of sacrificing the animal. Specimens were stored at 4 degrees Celsius until mechanical testing and kept moist with saline wetted gauze sponges until mechanical testing. All mechanical testing was performed using a MTS 858 Mini Bionix II testing system. An Axial-Torsional Load Transducer with an axial capacity of 15 kN and torsional capacity of 150 Nm was used at full scale. Data was recorded at 100 Hz to 4 decimal places and reported to 2 decimal places. Following all biomechanical testing, specimens were stored at -80 degrees Celsius until histological processing.

5.2.4 Histology Preparation

Specimens from Specific Aim #2 were prepared in the same manner as specimens from Specific Aim #1. For specifics, refer back to Section 3.2.2. Histological analysis is outside the scope of this thesis and will be the focus of further study.

5.2.5 Evaluation Methods

Qualitative evaluation in Specific Aim #2 had similar categories to Specific Aim #1 including apparent tissue adhesion, film formation, hematoma, infection, fluid filled bursa formation, and final fixation. In addition, the implants for Specific Aim # 2 were evaluated for apparent bone ingrowth and biomechanically tested for ultimate torque. At 6 and 12 weeks, all implants had excellent tissue adhesion, so the type of tissue was noted instead of the -. ±, +, ++ scale. For bone growth, the -, ±, +, ++ was used with the following criteria:

Qualitative bone ingrowth scale:
- (-) = no apparent bone growth
- (±) = callous growth
- (+) = callous growth with minimal bone growth
- (++) = excellent bone growth

In addition to qualitative results taken at the time of implant retrieval, the ultimate torque for each tibia was recorded as the measure of strength. The strength for each segmental tibia construct was expressed as a percentage of the ultimate torque of the contra lateral, unfractured tibia. Clinical observations for each animal were recorded as well.

5.3 Results (Aim #2) – Tibia

For Specific Aim #2, apparent bone growth was recorded in addition to all the previous categories from Specific Aim #1. Quantitative results were recorded for biomechanical strength for Specific Aim #2.

5.3.1 Surgical Procedure and Recovery

Two pilot animals were used to test and refine the surgical techniques and post-surgery husbandry care. The first pilot animal made it through surgery without any surgical complications but did not recover from anesthesia and therefore the animal's tolerance of a bilateral procedure could not be evaluated. The cause of death was determined to be prolonged operative time (> 5.5 hours) and a pancurium overdose. The second pilot animal made it through the surgical procedure and recovered from anesthesia but was euthanized 8 days following surgery because of prolonged signs of pain and intolerance. The animal would not walk on a regular basis and gastric functions never fully recovered following surgery. The tibia construct (Figure 19) was deemed inadequate and was redesigned to the construct described in Section 5.1.

The four control animal surgeries went as planned with no complications from fixation or infection. Even though goat #9 showed signs of ringworm, 2 small lesions, 10 weeks after surgery, this was deemed unrelated to the study and did not affect the results. Goat #9 was sacrificed at 12 weeks as planned.

Of the therapy animals, goats #11, #13, and #14, had no surgical complications during the procedure. NPWT was applied to the four therapy animals for 24 to 72 hours (until pump would no longer run due to pump error). Actual therapy durations are given in Table 9. Therapy times varied, from 23.35 hours to 70.79 hours, to determine how long negative pressure therapy could be maintained. Coagulation of blood in the tubing would eventually lead to loss of therapy turning the pump off. Therapy was discontinued when therapy could no longer be maintained. For future studies the therapy duration should remain consistent.

Table 9. NPWT durations for each animal

Animal Number	Therapy duration
Goat # 7, 6 week	control
Goat # 8, 6 week	control
Goat # 9, 12 week	control
Goat # 10, 12 week	control
Goat # 11, 6 week *(3 week due to infection)*	30.78 hrs
Goat # 12, 6 week *(3 week due to infection)*	70.79 hrs
Goat # 13, 12 week	28.14 hrs
Goat # 14, 12 week	23.35 hrs

Goat #12 had two surgical complications. The first complication was that the proximal cut of the osteotomy was not parallel to the distal cut. To correct this, an additional 2 mm segment of bone was taken out of the proximal end of the tibia to make the bone end better align with the proximal end of the implant. The second complication was insufficient fixation in the proximal screws of the slotted plate. This was resolved through the use of a cerclage wire around the proximal end of the plates. This animal was later euthanized 3 weeks following surgery because of a small infection in the proximal end of the surgical incision where the cerclage wire had created a poke-hole. The issue could not have been resolved without a follow-up surgery to correct the cerclage wire.

Goat #11 developed an infection approximately 2 weeks following surgery and was euthanized 3 weeks following surgery after an attempt at resolving the infection was unsuccessful. Upon dissection, a *pseudomonas aerugenosa* infection ran the length of the plate and had formed a white/yellow pus material beneath the incision line. This infection was confirmed through culture.

Goat #13 had two similar small lesions on her operative tibia 5 weeks after surgery. The lesions were found on the same day as those found on Goat #9 and were not wet. Later results showed that the lesions on Goat #13 were also from ringworm and unrelated to the study.

Overall, there were only two complications, one infection (Goat #12) and one surgical complication with the cerclage wire (Goat #11). The remaining six animals had no complications and made it to their respective term completion at either 6 weeks or 12 weeks.

5.3.2 Qualitative Results

The qualitative results for Specific Aim #2 taken at time of sacrifice are summarized in Table 10. Bone growth was improved among the control animals sacrificed at 12 weeks over the control animals at sacrificed at 6 weeks. No bone ingrowth was observed in the 2 therapy animals that were sacrificed at 3 weeks due to infection. White, fibrous tissue ingrowth was observed in all of the specimens with exception of the two infected implants. No hematoma or film formation was observed in any of the implants and only the severely infected specimen developed a bursa around the plates and implant. All implants maintained rigid fixation of the tibia construct. The following are the observations for each animal that were taken after euthanization and before mechanical testing.

Table 10. Qualitative results for Specific Aim #2

Animal	Bone Growth	Tissue Adhesion	Film Formation	Hematoma	Infection	Fluid Filled Bursa	Maintained Fixation
#7, 6 week control	±	Fibrous	No	No	No	No	Yes
#8, 6 week control	±	Fibrous	No	No	No	No	Yes
#9, 12 week control	+	Fibrous	No	No	No	No	Yes
#10, 12 week control	+	Fibrous	No	No	No	No	Yes
#11, 6 week therapy *(3 weeks due to infection)*	-	No/Pus	No	Yes	Yes	Yes	Yes
#12, 6 week therapy *(3 weeks due to infection)*	-	No	No	No	Yes	No	Yes
#13, 12 week therapy	±	Fibrous	No	No	No	No	Yes
#14, 12 week therapy	+	Fibrous	No	No	No	No	Yes

Supplemental information on each of the eight goats in Specific Aim #2.

Goat #7 was a 6-week control animal. There were no sign of infection and no hematoma, bursa, or film formation was observed. Bone growth was observed over the middle section of the slotted plate. There was callous formation around the distal end of the implant. Wear debris was observed at the second and third holes proximal from the implant and the most distal hole between the plate and bone.

Goat #8 was a 6-week control animal. There was no excess fluid or hematoma formation. Bone growth was observed over the proximal and distal ends of the slotted plate with callous formation around the proximal end of the implant. A narrow gap appeared between the distal implant/tibia interface on the radiographic image. No wear debris was evident at any of the screw holes.

Goat #9 was a 12-week control animal. There was no excess fluid or hematoma formation. Excellent tissue adhesion at 12 weeks, mostly white fibrous tissue, was observed. A

small Gap appeared on radiographic and fluoroscopic images between the proximal end of the implant and bone at the time of euthanization. No infection was evident clinically. Bone formation was most prevalent between the two plates and over the ends of the slotted plate on the caudal side of the tibia. The most proximal screw head in the slotted plate was covered by calcification and had to be removed using a ronjeur in order to remove the screw for mechanical testing. Underneath the calcification, wear debris was evident. Wear debris was also evident between the 12-hole plate and bone surface at the second most proximal hole. The debris did not appear to have caused any osteolysis but did affect a small area of the periosteum.

Goat #10 was a 12-week control animal. No excess fluid or hematoma formation was observed. We observed excellent fibrous tissue adhesion and apparent good union between proximal surface of the implant and bone. Goat #10 appeared to have more significant bone growth than goat #9. Bone growth was in a location consistent with the other 12-week control animal: around the slotted plate and between the two plates. Prevalent callous and bone formation was noted around the slotted plate with the two proximal screws and distal end of the plate covered by bone as well as an approximately 3 cm section at the proximal implant/bone interface. The distal screw head of the 12-hole plate was also covered by bone growth. The tibial tuborosity was noted as growing back out over the implant. A ronjeur was required to remove bone growth over the screw heads that were covered in order to remove the screws for mechanical testing. No infection was evident. There was evidence of wear debris in three screw holes in the 12-hole plate: the most proximal and the two immediately distal of the implant.

Goat #11 was originally a 6-week therapy animal but was sacrificed at three weeks because of a *pseudomonas aerugenosa* infection. After opening the incision, the infection ran the length of the plate and produced pale green pus. Minimal bone growth had taken place and no tissue adhesion. There was also a fluid filled bursa around the tibia construct, containing the infection.

Goat #12 was originally a 6-week therapy animal and was sacrificed at 3 weeks as a result of a surgical complication. The cerclage wire that was used proximal of the implant created an opening in the groin of the animal. There was no way to repair this hole without another surgery to correct the cerclage wire. As a result, there was little bone growth and tissue adhesion at the time of sacrifice. There were two hematomas, one over the implant and one proximal, where the cerclage wire was used.

Goat #13 was a 12-week therapy animal. There was some bone growth between the two plates and minimal topical bone growth over and around the slotted plate. Overall, less bone growth was observed in Goat #13 than in either of the 12-week control animals. There was complete fibrous tissue growth into the implant with no hematoma, fibrinoid film, infection, or fluid filled bursa formation. The implant maintained rigid fixation although the 4 screws in the slotted plate could be tightened one full rotation. There was evidence of wear debris in the most proximal hole of the 12-hole plate and was limited to the underlying soft tissue.

Goat #14 was a 12-week therapy animal. Bone growth was observed between the two plates and around the slotted plate including some topical bone growth. The amount of topical bone growth on top of and around the slotted plate appeared to be more than Goat #13 and less than Goat #9 and Goat #10. There was complete fibrous tissue ingrowth with no sign of a fibrinoid film, hematoma, infection, or bursa formation. No screws were found to be loose and no wear debris was evident.

5.3.3 Quantitative Biomechanical Results

Biomechanical results described below can be seen in Table 11. Goats #7 and #8, 6-week control animals, had percent torsion strengths of 3.64% and 0%, respectively. The implanted tibia of goat #7 could not be biomechanically tested because insufficient bone growth across the tibia/implant interface left the construct unstable after removal of the 12-hole and slotted plates. Goats #9 and #10, 12-week control animals, had percent torsion strengths of

7.99% and 5.93%, respectively. Goats #11 and #12, 6-week therapy animals, had to be sacrificed at 3-weeks because of infection and as a result, there was no stable fixation between the implant and bone. Because of unstable union at the implant/bone interface, the two implants were not biomechanically tested. Goats #13 and #14, 12-week therapy animals, had percent torsion strengths of 1.60% and 5.36%, respectively.

Table 11. Biomechanical results for Specific Aim #2

Animal #	Implanted Tibia Ultimate Torque (Nm)	Normal Tibia Ultimate Torque (Nm)	Percent Ultimate Torque of Implanted Tibia (%)
#7 (6 week control)	-	52.23	0 %
#8 (6 week control)	2.16	59.34	3.64 %
#9 (12 week control)	4.62	57.84	7.99 %
#10 (12 week control)	4.23	71.28	5.93 %
#11 (6 week therapy) *3 weeks due to infection*	-	-	- %
#12 (6 week therapy) *3 weeks due to infection*	-	47.06	- %
#13 (12 week therapy)	1.07	66.97	1.60%
#14 (12 week therapy)	2.94	54.81	5.36%

5.4 Discussion (Aim #2) – Tibia

Based on the results, there was a trend toward lower percent ultimate torque of the therapy group at 12-weeks (3.48% vs. 6.96%) although there are insufficient numbers to establish any level of significance. This is consistent with what was observed with respect to bone ingrowth. Bone growth was most prevalent over and around the slotted plate in the control and therapy animals and only after 12-weeks. Bone growth around the slotted plate is consistent with bone growing in the most stable part of the fixation construct. With the orthogonal arrangement of the two plates, the most stable segment of the interface is immediately along the line of the slotted plate. It is possible that the orthogonal plating technique was not stable enough across the

entire bone/implant interface to support bone growth across the interface. A finite element analysis of the orthogonal plating construct may reveal more regarding the stresses and strains that take place along the implant/bone interface.

Given that the percentage strengths of both 12-week therapy animals were lower than both percentage strengths of the 12-week control animals it appears that negative pressure therapy may inhibit bone growth. The trend is for worse bone growth with the use of negative pressure therapy. With only 2 animals in each group no statistical significance can be established and it is possible that the difference between these two groups can be explained by variance of the percentage strength within the population of each group.

One possibility as to why no positive effect of therapy on bone growth was observed could be explained by the relatively short duration of negative pressure therapy in relation to the time it takes to achieve bone growth and remodeling. Bone formation and remodeling takes up to six weeks in goats, but the negative pressure therapy could only be applied for a small fraction of this time (24-72 hours). Therapy duration varied within this range to determine how long negative pressure therapy could be applied in this manner. It is possible that a longer duration of therapy may have a greater impact on bone growth than what was observed in this study.

For the shorter term therapy group, the two 6-week therapy goats had to be euthanized early, at 3 weeks, because of infection. Because of this early termination, a comparison between the control and therapy groups cannot be made at this time interval. It is possible that NPWT increased the likelihood of infection, in which case developing a better method to maintain a sterile surgical site in the therapy group should be a priority moving forward. Similar to Specific Aim #1, maintaining a clean surgical site in a human application would not be as much of a concern as it is in an animal model.

As previously mentioned, a change from Specific Aim #1 that was found helpful during Specific Aim #2 to help with animal recovery following surgery was the use of Probios® following surgery. This probiotic helped these ruminants' digestive systems recover following surgery and is recommended as a regular part of any surgical procedure involving ruminants. The dosing was 10 g the evening of surgery and 5 grams two times a day until they were taken off buprenorphine.

Another topic of discussion is the method of application of negative pressure to the porous metal implant. Several methods were attempted during this study. In Specific Aim #1, a piece of sponge was used as a conduit to transmit the negative pressure from the pump through the incision down to the implant. A similar method is commonly used to transmit negative pressure along the leg underneath a cast to an open wound on the bottom of the foot. This method appeared to work for a time but eventually, the conduit sponge would lose contact with the implant surface or the exterior sponge or both.

In Specific Aim #2, for the second pilot animal, a hole was bored trough the implant along its length to insert a channel drain that would then distribute negative pressure through the implant. This appeared to be an effective method as fluid removal through the implant was observed during surgery before incision closure; however, the drain rapidly became clogged in approximately 24 hours and could only be removed and not replaced with this method. Also, the cost involved with creating the through hole so as not to clog the pores in the implant increased the cost of the implant beyond the feasibility of this study.

The third method, which was used for Specific Aim #2, was applying the T.R.A.C. Pad™ directly to the surface of the implant holding it in place with sutures. This proved effective and remained patent for 24-72 hours and could easily be removed when needed, again however, the T.R.A.C. Pad™ could not be replaced if necessary. Therapy could only be continued as long as

the tubing remained patent and there were no pump errors. Other than pump malfunctions, there was no way to monitor patency of the T.R.A.C. Pad™ and tubing. Pump errors and the V.A.C. Freedom® Alarm Trouble Shooting Guide can be seen in Appendix F.

Also discussed was the possibility of creating a transcutaneous fixture that attached to the implant so that tubing could be changed if clogged. This method could potentially allow for a longer application time of NPWT or the instillation of fluids, antibiotics, and growth factors to further improve healing.

There are many possibilities of how to apply negative pressure to distribute the therapy through the porous implant. Computer or bench top models may be a valid method to determine the optimal application of negative pressure therapy; however, the main dilemma we faced was clogging of the tubing due to coagulation. Any model would have to take blood coagulation into account in order to be useful for application to an *in vivo* model. Validation of the proper application of negative pressure therapy through a porous metal implant, either through a computer model, *in vitro* model, or combination, would be necessary before future *in vivo* studies should be done.

Chapter 6 Further Discussion

Chapter 6 Further Discussion

6.1 Limitations

There were several limitations in this study with the most obvious being the use of an animal model for a human application. An animal model was necessary to control variables such as defect size and location. For obvious reasons, such as the novelty of this technology and the inability to control variables, this research was inappropriate for human study at this point in the research. A caprine animal model was chosen for this study because of comparable bone size between goats and humans and its use by other research groups that are a part of AFIRM. The use of an animal model also introduced the additional issue of maintaining a clean wound that would not be as difficult of a concern to deal with in a human application. Humans understand that the wound needs to remain clean and that jumping around could damage fixation. Animals however, do not understand these issues.

Another limitation of this study was the low numbers of animals that were used. This was known upon initiating the study as it was planned to be a preliminary investigative look into the use of a novel combination of technologies to repair segmental defects. Small numbers became a further limitation when infection caused the removal of 1 animal from Specific Aim #1 and 2 animals from Specific Aim #2. If a technology combining negative pressure therapy with a porous metal implant were to be further developed, a larger study consisting of larger groups would be necessary to establish statistical significance of any benefits of the therapy. Another preliminary investigation would need to be done prior to a large animal study.

The inability to optimize the implant material for this application was another limitation of the study. We were unable to control certain characteristics of the material such as pore size and pore density because BIOFOAM™ is a proprietary material made by Wright Medical Technology, Inc. The BIOFOAM™'s material characteristics were optimized by Wright, but the only information regarding BIOFOAM™ that was made available was characteristic data from

internal company reports referred to on marketing and sales brochures. The original internal reports were not made available because it is against Wright Medical company policy. The characteristics of the material given on the sales brochures are given in Section 1.3.1.

Despite low numbers of animals, the inability to control the properties of BIOFOAM™, and the use of an animal model, this study established two useful models for the examination of negative pressure to induce bone and soft tissue ingrowth into porous metals. The purpose of this study was to establish proof of concept for later studies and provide animal models for future studies upon which to improve.

6.2 Future Directions

6.2.1 Future Directions – Specific Aim #1

From a study execution standpoint, the femur location used in Specific Aim #1a presented several problems for the application of NPWT. Negative pressure dressing changes, maintaining cleanliness of the underlying surgical site, and maintaining rigid fixation of the implant to the femur were all difficult. Because of these concerns, two animals were added to the original Animal Care and Use Protocol (ACUP) to pilot an alternative location on the iliac crest that was believed to provide several advantages over the femur. The iliac crest is further off the ground making dressing changes and inspection easier. In addition, the surgical site is further from the anus and does not touch the ground when the animal is lying down, keeping feces and urine away from the incision thereby decreasing the likelihood of contamination and infection. The animal also cannot lay or fall on the implant, decreasing the likelihood of losing rigid implant fixation. Lastly, the iliac crest provides an anchor point for many of the surrounding muscles and tendons, which inherently means less movement of the soft tissue over the implant as soft tissue tries to grow into the implant. Movement of the surrounding soft tissue across the implants may have contributed to the formation of fluid filled bursas around some of the implants in Specific Aim #1. Because of these benefits, it is recommended that the iliac crest be used for further soft

tissue, non-load bearing studies. A more detailed description of the outcomes of the two animals that were piloted using the iliac crest can be found in Chapter 5.

Also deserving further investigation is the method in which negative pressure is applied to a sub-dermal porous implant. The method used in Specific Aim #1 utilizing a transmittance sponge may be improved upon and deserves further investigation before further *in vivo* testing.

6.2.2 Future Directions – Specific Aim #2

Looking long term, a permanent, non-degradable, metallic implant such as the BIOFOAM™ implant used, may not be ideal for repairing large, critical segmental defects. A porous metal was used in this study to allow for focus of the effects of negative pressure therapy in such an application but a biodegradable material would be more desirable long term. While there are no known materials currently available, the development of bioresorbable material to be used in an internal fixation device would be beneficial. Such a material would need to provide the proper rigidity to initially stabilize a defect, the porosity necessary for the application of negative pressure to the surrounding tissue, and the ability to be degraded and absorbed by the body over time, allowing the eventual complete takeover of bone growth. A standard implant size could be easily used in a surgically created bone defect such as an osteosarcoma osteotomy; however, such an implant is not easily adapted for the irregular segmental defects created by high impact trauma. Unlike metals, if a material had the capability of being molded on site, it may also be able to better adapt to non-uniform defects like EWIs.

A plating method of fixation was originally chosen for Specific Aim #2 so as not to interrupt the porous titanium implant, but the double plating technique may not provide rigid enough fixation for primary healing and bone growth to occur across the implant/bone interface. An FEA analysis of the two plate technique that was used would be beneficial to calculate the stresses and strains. A better alternative to the two plate technique may be the use of an intramedullary (IM) nail. This would disrupt the porous implant but maintaining even rigid

fixation may be more important for primary bone healing across the implant interface. An IM nail could be combined with a biodegradable porous implant. Mechanical testing and/or FEA analysis to verify the IM nailing technique is recommended before further *in vivo* testing.

Lastly, similar to the future directions for Specific Aim #1, the optimization of the method of applying negative pressure therapy to a sub-dermal, porous metal implant in order to achieve longer duration of therapy deserves further investigation. Several methods, including the two used in this study, were discussed in Section 5.4. NPWT is currently mostly limited to surface applications and little is known about its potential in closed, sub-dermal wounds. Finite element analysis, computer models or *in vitro* models offer potential methods through which to optimize the use of negative pressure in a sub-dermal application before further *in vivo* testing. Applying negative pressure therapy to bone for a longer duration may be necessary to have an effect on bone growth. The durations that were achieved in this study (24-72 hours) were short relative to the time it takes bone to form (6 weeks).

Chapter 7 Conclusions

Chapter 7 Conclusions

This study establishes the use of caprine models to evaluate soft tissue and bone ingrowth into large porous implants. While the greater trochanter of the femur sufficed in Specific Aim #1 for the purpose of this study, it is recommended based on two pilot animals, that future studies use the iliac crest as the implant location for future non-load bearing soft tissue studies. The iliac crest provides a more sanitary and easily accessible location over the greater trochanter with less likelihood of the goat damaging fixation of the implant and less movement in the surrounding soft tissue.

In all six animals of Specific Aim #1, gross examination showed improved tissue adhesion to the implant treated with NPWT when compared to the control despite the level of negative pressure of duration of study period. While numbers are insufficient to establish statistical significance, this study provides preliminary evidence that negative pressure may induce soft tissue ingrowth into a large porous titanium implant that could be beneficial for future investigation.

Specific Aim #2 established a unilateral model for the future study of segmental defects and methods to repair such injuries. A benefit of negative pressure therapy on bone growth from the therapy durations used in this study was not able to be established; however, longer durations of therapy may have a greater impact on bone growth. For future studies, it is recommended that all study terms be carried out to at least 12 weeks in order to see some bone remodeling occur. In addition, mechanical strength of an intramedullary nail and validation of the best method of application of negative pressure therapy should be evaluated through either a computer model or *in vitro* model before future *in vivo* studies are carried out.

This study establishes two *in vivo* caprine models that can be used to evaluate a device combining NPWT with a porous implant that could be used in repairing large injuries with

defects in soft tissue such as people suffering EWIs, bone cancer, or other traumatic injury and establishes two animal models for evaluation of such a device.

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[1-58]

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Appendix A: Medication List

Table 12. Medications used with usage and dosing information

Drug	Class of Drug	Dose	Route	Duration of Treatment
cephalaozine	antibiotic	15 mg/kg	SQ	once preop
xylazine	anesthetic	0.03 mg/kg	IM	once for surgery, once each time for sedation
ketamine	general anesthetic	5 mg/kg	IM	once at induction
ketamine	general anesthetic	10 mg/kg	IM	weekly as needed for x-ray
diazepam	benzodiazepine derivative	0.4 mg/kg	IV	once at induction
morphine	narcotic	0.1 mg/kg	Epidural	once at induction
flunixin	nSAID coxib	1 mg/kg	IV	once at induction
buprenorphrine	narcotic/analgesic	0.005 mg/kg	IM	once 4 hrs postop and BID for 72 hours
beuthanasia-D special (sodium pentobarbital)	barbiturate	90 mg/kg (0.22 mL/kg)	IV	once
lidocane	amide/anesthetic	1 mg/kg	SQ	once
pancurium	paralytic	0.06 mg/kg	IV	once
isoflurane	general anesthetic	1-2%	Inhalation	until disconnected
oxytetracycline	broad spectrum polyketide antibiotic/fluorochrome	25 mg/kg	IM	week 4 and week 10
calcein green	fluorochrome	20 mg/kg	SQ	week 2 and week 8
xylenol orange	fluorochrome	90 mg/kg	SQ	week 6
fentanyl	analgesic	50 mgc/hr/3 days	TD	once, as needed

Appendix B: Histology Embedding Protocol

Table 13. Step by step embedding protocol in table format

Solution	Time	Temperature	Additional Treatment
Neutral Buffered 10% Formalin	24 hours	Room	Vacuum 6 hours Agitate 18 hours
Neutral Buffered 10% Formalin	24 hours	Room	Vacuum 6 hours Agitate 18 hours
Neutral Buffered 10% Formalin	24 hours	Room	Vacuum 6 hours Agitate 18 hours
100% Acetone	24 hours	Room	Vacuum 6 hours Agitate 18 hours
100% Acetone	24 hours	Room	Vacuum 6 hours Agitate 18 hours
50% Spurr Resin: 50% Acetone	24 hours	Room	Vacuum 6 hours
75% Spurr Resin: 25% Acetone	24 hours	Room	Vacuum 6 hours
100% Spurr Resin	24 hours	Room	Vacuum 6 hours
100% Spurr Resin	24 hours	Room	Vacuum 24 hours
Embed in 100% Spurr with Activator	72 hours	Room	Vacuum 72 hours
Move to refrigeration	11 days	4 °C	-
Move to incubator	Until hard (~72 hours)	45 °C	-

Appendix C: Histology Sectioning and Grinding Protocol

Table 14. Histology grinding protocol

Paper Grit	Final section thickness
120	300 µm
180	220 µm
240	200 µm
320	180 µm
400	150 µm
600	100 µm

Appendix D: H&E Staining Protocol

Table 15. H&E staining protocol

Step	Solution Medium	Time
1	Epoxy Resin Removal Kit	30 minutes
2	100% Anhydrous Alcohol	3 minutes
3	100% Anhydrous Alcohol	3 minutes
4	80% Alcohol	2 minutes
5	Tap water rinse	30 seconds
6	Deionized water rinse	30 seconds
7	Hematoxylin	1-5 minutes
8	Tap water rinse	1 minute
9	Define solution	30-90 seconds
10	Tap water rinse	1 minute
11	Blue Buffer solution	30-60 seconds
12	Tap water rinse	30-60 seconds
13	80% Alcohol	1 minute
14	Alcoholic Eosin	30-90 seconds
15	80% Alcohol	2 minutes
16	100% Anhydrous Alcohol	3 minutes
17	100% Anhydrous Alcohol	3 minutes
18	Xylene	5 minutes
19	Xylene	5 minutes
20	Xylene	5 minutes

Appendix E: Complete List of Surgical Supplies (not all shown in pictures)

Instruments

- 2 - needle holders
- 2 - pairs of dissecting scissors
- 2 - pairs of suture scissors
- 2 - baby Hohmann retractors
- 2 - army/navy retractors
- 1 - set of plate benders
- 2 - screwdrivers
- 2 - scalpal handles
- 6 - vascular clamps
- 1 - Rongeur
- 1 - 30 mL irrigation syringe
- 2 - small metal bowls
- 6 - towel clamps
- 2 - saw blades
- 3 - drill bits
- 1 - drill guide
- 1 - depth gage
- 2 - Verbruge clamps
- 1 - power drill
- 1 - power drill cord
- 1 - drill chuck with key
- 1 - Screw cutters
- cerclage wire
- 1 - Cobb
- 1 - osteotome
- 1 - mallet
- 1 - Weitlaner Retractor
- 1 - small tissue forceps
- 1 - medium tissue forceps
- Cutting guide
- 2 - stainless steel ¾", 8-32 machine screws
- 2 - stainless steel ½", 8-32 machine screws
- 2 - porous metal tibial implants (Wright Medical Technology, Inc.; Arlington, TN)
- 20 - 4 by 4 gauze sponges
- 2 - 30 by 30 impermeable drapes

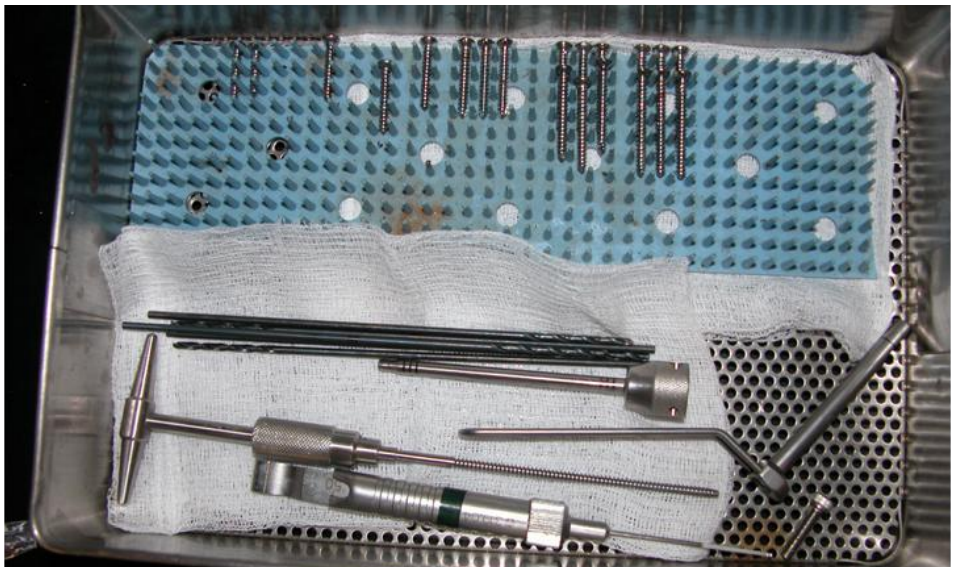
Purchased from Veterinary Orthopedic Implants, St. Augustine, FL

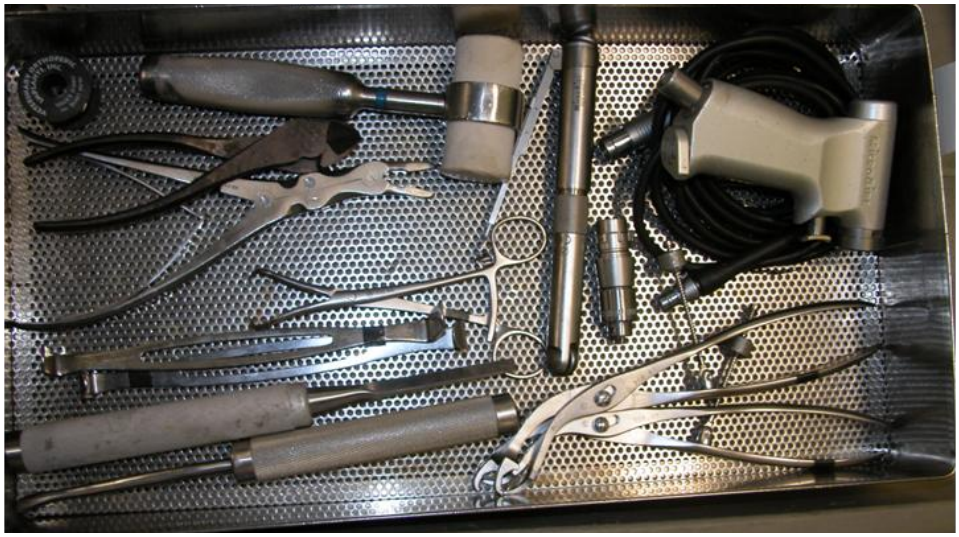
- 1 - tap
- 1 - tap guide
- 1 - tap handle
- Bicortical screws
    - 10 - 18mm 3.5 mm dia. screws
    - 10 - 20 mm 3.5 mm dia. screws
    - 10 - 22 mm 3.5 mm dia. screws
    - 10 - 24 mm 3.5 mm dia. screws
    - 10 - 26 mm 3.5 mm dia. screws
    - 10 - 30 mm 3.5 mm dia. screws
    - 10 - 34 mm 3.5 mm dia. screws
- 2 - 10 mm 12-hole compression plates
- 2 - 10 mm 8-hole slotted plates
- 2 - 10 mm 12-hole compression plates
- 2 - 10 mm 8-hole slotted plates

Other Consumables

- Sterile gown
- Ioban Drape
- Impermeable Extremity Drape
- 3-0 Vicryl suture
- 2-0 Vicryl suture
- 1 mL Heparin (10,000 units/mL)
- 1 - syringe and needle for heparin
- 500 mL irrigation saline
- V.A.C. dressing
- V.A.C. Freedom® pump
- channel drain
- drain bulb
- face masks
- shoe booties
- sterile surgical gloves
- sterile towels
- Veet®
- No. 15 scalpel blades
- Power unit for drill
- Camera

Figures 24, 25, 26. Surgical Kit





Appendix F: V.A.C. Freedom® Alarm Troubleshooting Quick Reference guide

Figure 27. V.A.C. Freedom® Alarm Troubleshooting Quick Reference guide page 1.

vac freedom

V.A.C. Freedom® Alarm Troubleshooting Quick Reference

Quick reference for the delivery of prescribed therapy.

The V.A.C.® Freedom Therapy System has been designed to deliver the proven benefits of V.A.C.® Therapy in an easy-to-use system that fits patients' active lifestyles. Understanding its functions and being able to identify and resolve potential problems is essential to providing effective therapy.

Customers needing technical assistance, please call KCI at 1-800-275-4524 to troubleshoot in real-time over the phone 24 hours a day.

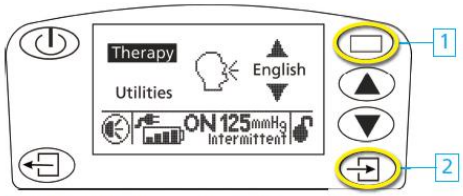
Detecting and resolving common V.A.C.® Freedom Therapy alarms. To be used in conjunction with device *User Manual*.

System Will Alarm If...	Action...
Canister is full or tubing blocked	This alarm will sound if the canister is full or if the tubing is kinked or blocked. Replace canister if full.
Canister is missing or not fully engaged	Ensure V.A.C. Freedom® canister is present and fully engaged.
A leak is detected in the dressing	You may hear a whistling sound indicating air is entering into the drape. Often, the leak is around the tubing. Caregiver should pat around drape to check for leaks. If leak is detected, patch the leak with extra drape. (Leak can prematurely run battery down.)
Battery is low	Recharge battery by plugging the system into the wall outlet.
Therapy not activated (System is on but therapy is not activated.)	Press POWER to turn unit off, then press again to turn POWER and unit on. If the therapy remains off, see the back side of this card.

If active bleeding develops suddenly or in large amounts during V.A.C.® Therapy, or if bright red blood is seen in tubing or in the canister, immediately stop V.A.C.® Therapy (leave dressing in place for physician to remove), take measures to stop the bleeding, and seek immediate medical assistance. Contact patient's attending physician, home health agency or wound care center for further medical assistance, or local emergency number (i.e. 911).

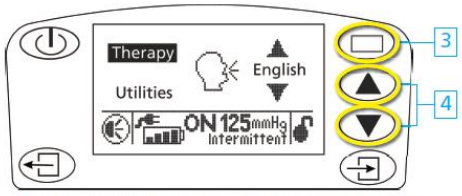
Figure 28. V.A.C. Freedom® Alarm Troubleshooting Quick Reference guide page 2.

If necessary to reset Therapy to the original settings in the event of an alarm, do the following:



1. Highlight Therapy on the main screen by pressing the **Select** button (top right button with picture of box).

2. Then press the **Enter** button (bottom right button with picture of arrow pointing into box).



3. Next, highlight Therapy by pressing the **Select** button (top right button with picture of box).

4. Once Therapy is highlighted, press the **UP** or **DOWN** arrow buttons to turn the Therapy ON (the down arrow button will set Therapy to Continuous and the up arrow button will set Therapy to Intermittent).

Note: If you are unable to reset Therapy to the original setting, contact the patient's attending physician, and/or your local KCI Clinical Consultant.

Note: Do not change the Therapy mode to a setting that is different from the original setting without first consulting with the patient's attending physician.

Note: This card is a quick reference source specifically for device alarm conditions and Therapy reset. It is not a substitute for device Instructions For Use. Please consult instructions provided with the device for product use and detailed information.

For more information about V.A.C.® Therapy
call KCI at 1-800-275-4524
or visit us online at www.kci1.com.

Note: Specific indications, contraindications, warnings, precautions and safety information exist for KCI products and therapies. Please consult a physician and product instructions for use prior to application.

KCI

CAUTION: Federal law restricts this device to sale/rental by or on the order of a physician.

Appendix G: Post surgery record forms

Figure 29. Weight record form.

Animal #_____

Weight Record

Initial Weigh	Date	Comments

Pre Surgery Weight	Date	Comments

Weekly weigh-in post op		Date	Comments
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			

Figure 30. Medication record form.

Animal #_____

Medication Record

Date (mm/dd/yyyy)	Pre Surgery Medications	Justification

Date (mm/dd/yyyy)	Post Surgery Medications	Justification
	Buprenex or Fentanyl	Post Surgery Analgesic 1
	Buprenex or Fentanyl	Post Surgery Analgesic 2
	Buprenex or Fentanyl	Post Surgery Analgesic 3
	Buprenex or Fentanyl	Post Surgery Analgesic 4
	Buprenex or Fentanyl	Post Surgery Analgesic 5
	Buprenex or Fentanyl	Post Surgery Analgesic 6

Date (mm/dd/yyyy)	Enthanization		Justification (circle)
	Beuthansia-D	mL	Protocol or Adverse Event

Figure 31. Gait progress form

Animal #_____

Gait Progress

Scale for Gait monitoring

0 = not used at all

1 = supported incidentally

2 = loaded in a standing position and incidentally while walking

3 = loaded in a standing position and while walking but with a limp

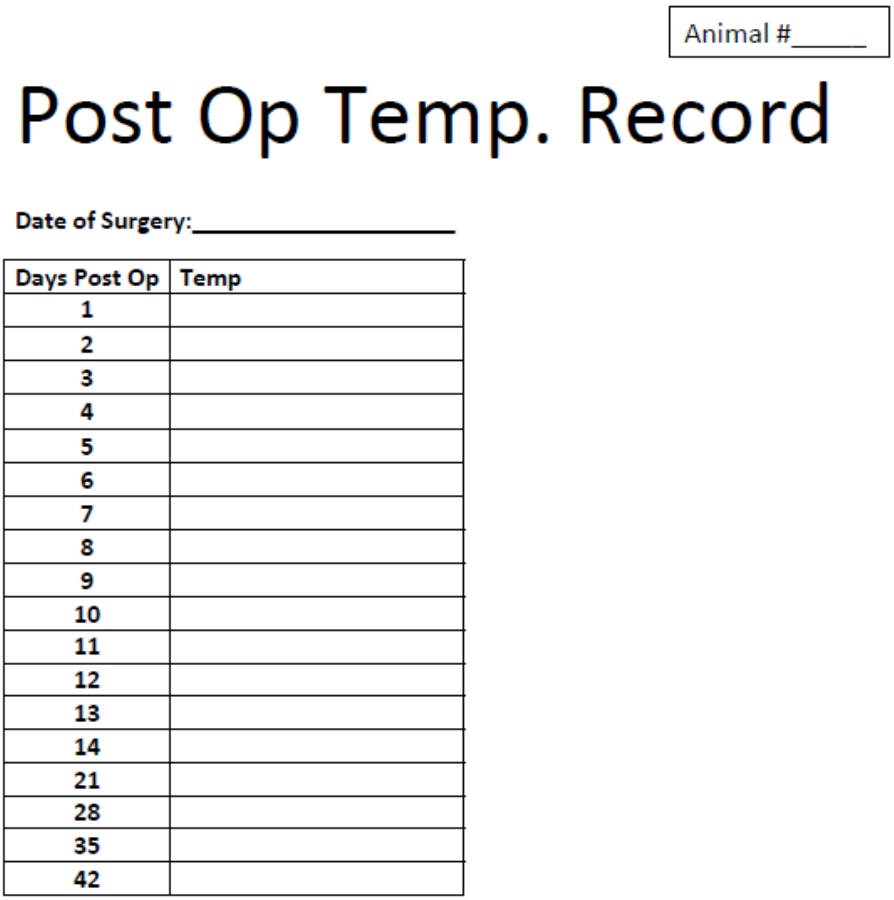
4 = normal walking and standing pattern

Date of Surgery:_____

Days post op	Gait
1	0 1 2 3 4
2	0 1 2 3 4
3	0 1 2 3 4
4	0 1 2 3 4
5	0 1 2 3 4
6	0 1 2 3 4
7	0 1 2 3 4
8	0 1 2 3 4
9	0 1 2 3 4
10	0 1 2 3 4
11	0 1 2 3 4
12	0 1 2 3 4
13	0 1 2 3 4
14	0 1 2 3 4
15	0 1 2 3 4
16	0 1 2 3 4
17	0 1 2 3 4
18	0 1 2 3 4
19	0 1 2 3 4
20	0 1 2 3 4
21	0 1 2 3 4

Days post op	Gait
22	0 1 2 3 4
23	0 1 2 3 4
24	0 1 2 3 4
25	0 1 2 3 4
26	0 1 2 3 4
27	0 1 2 3 4
28	0 1 2 3 4
29	0 1 2 3 4
30	0 1 2 3 4
31	0 1 2 3 4
32	0 1 2 3 4
33	0 1 2 3 4
34	0 1 2 3 4
35	0 1 2 3 4
36	0 1 2 3 4
37	0 1 2 3 4
38	0 1 2 3 4
39	0 1 2 3 4
40	0 1 2 3 4
41	0 1 2 3 4
42	0 1 2 3 4

Figure 32. Temperature record form.



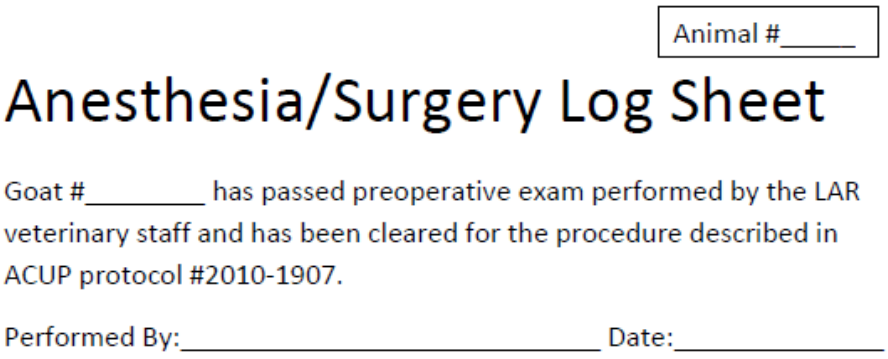
Animal #_____

Post Op Temp. Record

Date of Surgery:_____

Days Post Op	Temp
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
21	
28	
35	
42	

Figure 33. Anesthesia/surgery records form



Animal #_____

Anesthesia/Surgery Log Sheet

Goat #_____ has passed preoperative exam performed by the LAR veterinary staff and has been cleared for the procedure described in ACUP protocol #2010-1907.

Performed By:_____ Date:_____

Anesthesia Notes:

Figure 34. Therapy record form.

Animal #_____

VAC Therapy Record

Date	Pump #	Actual Start Time	Total Therapy Hours @ Start	Actual Time for check	Total Therapy Hours @ Time of Check